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BIOLOGICAL CONTRIBUTIONS

*A Collection of Essays and
Research Articles Dedicated to*

JOHN THOMAS PATTERSON

on the Occasion of his Eightieth Birthday

Edited by Marshall R. Wheeler

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**BIOLOGICAL
CONTRIBUTIONS**

The benefits of education and of useful knowledge, generally diffused through a community, are essential to the preservation of a free government.

SAM HOUSTON

Cultivated mind is the guardian genius of Democracy, and while guided and controlled by virtue, the noblest attribute of man. It is the only dictator that freemen acknowledge, and the only security which freemen desire.

MIRABEAU B. LAMAR

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J. T. P. : An Abstract

*Knowing you
As teacher, colleague, friend,
Has been to drop the plumbline
Of integrity beside the words
And find them true.*

*That just acclaim
The world of science has given unstintingly
Rests well on you
To whom the day's work means more
Than fame.*

*The years will tell
Your best reward—a growing company
Who teach and work more knowingly
Because you worked and taught
So well.*

. . . . LINDA WHARTON McDANALD
Lamarque, Texas



JOHN THOMAS PATTERSON

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Professor Patterson with a group of his Ph.D. students, taken on the occasion of his 80th birthday. Left to right, seated: Elsie Bodenmann, Meta Suche Brown, Johanna Blumel, Carl G. Hartman, Professor Patterson, Linda Wharton McDonald, Sarah Bedichek Pipkin, Ola Johnston. Standing: William K. Baker, T. C. Hsu, Marshall R. Wheeler, G. W. D. Hamlett, James F. Crow, George H. Mickey, Dean R. Parker, Walter J. Burdette, Wilson S. Stone, William B. Heed, L. E. Rosenblad, Robert P. Wagner, Russell W. Cumley. Austin, Texas, November 3, 1958.

Introduction

JOHN THOMAS PATTERSON

A Tribute on His 80th Birthday (November 3, 1958)

C. P. OLIVER

Chairman, Department of Zoology,
The University of Texas, Austin

Very few scientists have the opportunity to recall a half of a century of past experiences and activities during which they played a part in developing a field of science. Any man must have a feeling of satisfaction when he can see how much his own work has meant to a science. John Thomas Patterson is one of those few, fortunate scientists.

Fifty years ago J. T. Patterson began his scientific career at the relatively new University of Texas. The biological research at the school was not, at that time, influencing scientific opinions to any great extent. Actually, the Department of Zoology (known as a School in those days) was only about ten years old; and its predecessor, the School of Biology, had been organized less than ten years earlier. For fifty years, Professor Patterson continued his activity as a member of the faculty of the school. By his own Herculean efforts as an investigator and by following a sound policy as an administrator, Patterson has been responsible for developing a department which has gained international recognition for its research in biology.

Soon after he came to the University of Texas, Professor Patterson began a teaching program that attracted the attention of the more alert advanced students. They were attracted to him by his friendliness, scholarly attitude, and enthusiasm. Science was truly a big part of his everyday life. He came to the laboratory early and he stayed late. His students developed a devotion for their teacher and the devotion was lasting. Those students are justly proud of their associations with their teacher. They have a right to their pride. They cannot, though, keep all of the feeling and elation for themselves. The man's heart was big enough to take in more than his own students. We who merely worked in the same laboratory with Professor Patterson, as students or colleagues or visitors, have also enjoyed the man's warmth of friendliness, his spirit of cooperation, and his words of encouragement in our work. Either by his spoken word or in his thoughts, every one of us refers to the likeable "bundle of enthusiasm" as "Pat."

On November 3, 1878, John Thomas Patterson was born near Piqua, Ohio.

He received the Bachelor of Science degree from the College of Wooster, Ohio, in 1903. Patterson then joined the faculty of Buena Vista College in Storm Lake, Iowa, where he was Professor of Biology during 1903–1905. He then entered the University of Chicago to complete his university education. In 1908, the University of Chicago awarded Patterson the Doctor of Philosophy in Zoology, *summa cum laude*.

In 1908, the new Dr. J. T. Patterson accepted an invitation of the young University of Texas to join its staff as an Instructor of Zoology. He was one-half of the departmental faculty, the other being Dr. H. H. Newman who came to the department as Head also in 1908. Dr. Patterson was promoted to Adjunct Professor in 1911 and to Professor in 1913. He was elevated to the position of Distinguished Professor when that rank was established by the University in 1937. With his retirement in 1955, he was named Emeritus Professor.

Two predecessors (William M. Wheeler and T. A. Montgomery) transmitted to Patterson and Newman an atmosphere for research in the institution. The new staff, though, began to develop experimental research in the laboratory. They set out purposely to develop strong undergraduate courses, few in number, but selected to meet the needs of a general education as well as to interest students in and to prepare them for graduate work.

In 1911, Professor Newman resigned and Professor Patterson became Chairman of the School of Zoology. He remained Chairman for the term 1911–1913 and again for the period 1916–1925.

This appointment had a strong and lasting beneficial effect on Zoology at the University of Texas. He established the policy of electing new staff members who showed promise and who were especially trained to teach and direct some particular phase of Zoology. Courses were kept to a minimum number to give the new teacher an opportunity to carry on productive research. Facilities as well as time were made available for scholarly productivity. Professor Patterson became Director of Research in Zoology in 1928 and retained the position until his retirement in 1955. In this position he was able to help promote active research programs of his colleagues.

As a teacher and as a researcher, Professor Patterson has had a profound influence upon the development of genetics and cytology. When he first accepted appointment with the University of Texas, Patterson taught cytology, physiology, and comparative vertebrate anatomy. In 1911, following Newman's resignation, he assumed the duties of teaching heredity and genetics. As new staff members were added to handle each his own specialty, Patterson gave up courses to them and took over other duties. During his period as a teacher in the department, Patterson has taught twenty-six different courses.

The influence Patterson has had on research in genetics and cytology is the result of his own productivity and the effect he has had on the research of his colleagues. His intellectual curiosity led him to ponder over unknown recesses in the exoskeleton of biology. Then experiments were designed to explore the unknown. Once that had been done, his whole being was poured into the investigations, regardless of the subject, until an answer appeared. Collaborators in the department permitted a broader scope of the research. No pressure was

exerted, though, to get the staff to work in any particular field. Each was permitted to do what he preferred to do. But the spirit of the department was one of cooperation, an example set by Patterson. Research reports came rapidly from the Department of Zoology and represented in many instances a community effort to open up new avenues for the study of genetics and cytology.

Professor Patterson's early research was in embryology and polyembryony. He gave us the story of the monozygous origin of the quadruplets produced by the armadillo. After the discovery by his colleague, H. J. Muller, that mutations can be induced by irradiation, Patterson began an active research program in genetical problems. This included studies in the effects of irradiation and the use of material so produced to investigate other biological problems. He determined that mutations can be induced in somatic cells of *Drosophila*. With students and colleagues, he established the relationship between chromosome parts and sex determination in *Drosophila*, and showed that mutations can be reversed.

An interest in evolution and genetics led Patterson to studies of the species relationships in the genus *Drosophila*. Intensive collecting excursions in the field became necessary. This study began in 1933 and sample collections were made of wild types of *Drosophila* in Texas and surrounding areas. Results were so promising that in 1938 a large and definite program was organized. Many trips were made. The interest was great. Any one who made an automobile trip with Patterson to collect specimens might wonder whether or not he would get around the next curve. But he was confident that the trip would end with a mass of material on hand and enough work to last the laboratory for some time. Because of the success in the near areas, collecting trips were made to other parts of the United States and to areas to the south. The laboratory became a cooperative caldron for studies of taxonomy and species relationships. In order to report the investigations in more detail, a series of bulletins was published by the University of Texas Press. These "Studies in the Genetics of *Drosophila*" were edited by Patterson until his retirement from the faculty. In the bulletins are descriptions of species, reports on hybridization tests, relationships between species as shown by genetical and cytological tests, isolating mechanisms, and other genetical studies. In 1952, a book entitled "Evolution in the Genus *Drosophila*" was authored in collaboration with W. S. Stone. The latest paper by Patterson was published in 1957 in the University of Texas Publication 5721.

Patterson has gained the recognition of and has been honored by his colleagues in the United States and in foreign countries. He was elected to membership in Sigma Xi by the Chapter at the University of Chicago. That school and, later, the College of Wooster elected him to Phi Beta Kappa. He received an honorary Doctor of Science from the College of Wooster. In 1913, he was elected to membership in the Town and Gown Club in Austin, Texas. A number of professional societies have elected him to be their president; they are, the Texas Chapter of the Society of Sigma Xi (first president), American Society of Zoologists (1939), Society for the Study of Evolution (1947), and Genetics Society of America (1954). In 1956, he was made honorary member of the Genetics Society of Japan. In 1941, Patterson was elected to membership in the National Academy of Sciences. He received the Daniel Giraud Elliot Medal in 1951 from the

National Academy of Sciences for his study of isolating mechanisms reported in 1947.

Students who received their training under Professor Patterson's direction remain loyal to him. Evidence for this is definitely shown by the number who attended the eightieth birthday celebration. As one who was not Patterson's student, I am permitted to say that the scientific success of his students shows the soundness of his training in the ways of science. The enthusiasm, devotion to science, and cooperativeness rather than strict competition and individualism leached down to them and became permanently incorporated.

From 1913 through 1952 Patterson was major adviser for 31 successful M.A. candidates. The first student to be granted the Ph.D. degree under Patterson (and by the University of Texas) received the degree in 1915. He has had twenty-nine of his candidates receive the Ph.D. degree from 1915 through 1955.

Professor Patterson's eightieth birthday offered a happy occasion for his colleagues, former colleagues, and students to express their gratitude and esteem. The contributions in this anniversary volume are offered as a small tribute to this remarkable man.

The Making of a Scientist

CARL G. HARTMAN¹

North Plainfield, New Jersey

It was Professor Patterson (hereinafter called "Pat") who invited me in 1912 to join his Department as Instructor. As a result he was "stuck" with me for thirteen years, when I joined the Department of Embryology of the Carnegie Institution of Washington in Baltimore. In those thirteen years I learned much that influenced my later career. Incidentally, I also enrolled as Pat's student, and being successful in winning a Ph.D. degree, I have the honor of holding the first doctorate conferred by the University of Texas.

In an attempt to explain how I came to be a scientist, I must furnish a somewhat detailed background. I may, at the outset, state categorically that I never consciously said to myself: "I am going to be a scientist." I have sympathy for the High School youth who cannot make up his mind, in view of the fact that I was 33 before I reached this stage myself! I have, in fact, been a drifter, partly for reasons beyond my control (or so I imagined), always following the next line of endeavor that interested me at the time and which was often quite remote from natural science. However, it will be apparent from the following that any young man with ambition, energy, a fair IQ, and good health can rise well up the ladder of accomplishment in science if he has a mind to do so.

My tendency to take up various lines of employment was perhaps a matter of expediency, but it shows that I had not set my goal early, certainly not before I had reached the age of 32 when I was lucky enough to be able to join Pat's competent staff at the University of Texas. Rumor has it that Pat, before he offered me the job, wrote my former Professor, Dr. W. M. Wheeler, asking whether he thought that I would "stick" this time. I stuck.

It all began back in my early days in Iowa, my native state. Not getting along with the male teachers in the German-American parochial school, my mother and my preacher father (D.D., M.D.) sent me to the public school where I entered the eighth grade. In the first two years of the Clinton, Iowa, High School I had the only pre-university science course, physics. This was well taught, I think, for it interested me greatly and for some years I kept my notebook containing drawings of all the apparatus the school possessed.

Since my father believed that a classical education was the proper preparation for any career, I was naturally required to begin Latin early. My High School studies were no challenge, as I remember them, which gave me the opportunity to read most of the fiction in the small school library. I also had a bout with several hundred dime novels, the reading or even possession of which was strictly prohibited. I recall Horatio Alger, Oliver Optic, and Henty, wept at the death of the Last of the Mohicans and rejoiced with Tiny Tim. From fiction I moved into history. No popular science books such as the youth of our day have available had been written—unless one excepts those of Jules Verne.

¹ B.A., 1902; M.A., 1904; Ph.D., 1916, The University of Texas.

My most profitable and awakening year was spent at Wartburg College, a German-Lutheran pre-theological preparatory school. I had not the slightest thought of becoming a minister, but the cost of tuition and board (\$250 a year) was in keeping with my father's ministerial income (even when supplemented with some fees from his small medical practice) and a family of seven children plus two adopted ones! At Wartburg, Greek and Latin were stressed—to be translated into German. In fact, all of my studies were in German: German literature and rhetoric, church and secular history, and weekly essays. There was no Math. and no study at all in English. The professors were graduates of German Universities and Gymnasia and were "tough hombres." For the first time in my life I really learned to study. Something like their demands, which obtain generally in nations with which America is in competition, is now needed in this country. The year at Wartburg was most valuable to me, as it gave me a command of German almost equal to that of English. This later proved a great asset in my future research work.

At the end of the Wartburg session I took the entrance examination at the University of Iowa and was admitted, probably because of my fluency with the languages. Of my year as a green Freshman I recall only the courses in English and mathematics. But the University, where my father had taken his M.D. degree ten years previously, seemed to me an imposing institution. The summer between Wartburg and the University I spent as a carpenter's assistant in LaPorte City, Iowa, my parents having moved to Texas and there being no funds for my traveling back and forth between Iowa and Texas just for a vacation.

At the end of the 1896-7 session at the University of Iowa it became necessary to have my eyes looked after. The famous ophthalmologist, Dr. Schneider of Milwaukee, diagnosed the trouble and referred me to Dr. Hilgartner in Austin, Texas, near which city I was to live for the next three years. As will be noted below, my eye trouble greatly affected my career, at least for a number of years.

Eye rest was prescribed. What better place for this than a farm? The next three years I spent on a farm at Pflugerville, 13 miles from Austin and 10 miles from Round Rock, and from the nearest railroad—*i.e.*, before the M.K. & T. came through Pflugerville and destroyed its rusticity. My apprenticeship was soon over and I became a full-fledged farm hand at \$3.50 a week plus room and board. A city boy suddenly found himself at a rugged calling. All the activities, including five meals a day, were new to me, from work in the cotton and corn fields to driving a few thousand head of sheep across the prairie. My first initiation to surgery and endocrinology was participating in the castration of mature boars. and sterilizing the wound with a handful of mud from the nearby pond or "tank" with its coating of algae—and its content of antibiotics. I should explain, perhaps, that a boar has little market value until his scent glands atrophy as a result of castration.

One day the local school board urged me to get on my horse, ride to Austin, and take the examination for a teacher's certificate. This I did, bringing back a temporary certificate (which was later made permanent). When, many years later, I was head of the Department of Zoology at the University of Illinois, and since there was certificate reciprocity between Texas and Illinois, I was the only member of that large and competent faculty who was "certificated" to teach in

the public school of that state! But for two winters at Pflugerville I taught the local school of 50 pupils in eight grades for \$50 a month.

I did pick up a little science on the farm. The Texas prairie produced many handsome flowers in gorgeous profusion—roadways lined with the pink evening primrose, the prairie blanketed with gaillardias for a thousand miles. I wished to get better acquainted with them, so I secured a copy of Gray's *Botany* and learned the technical terms necessary to run the keys to the flowering plants ("repand margin," "mucronate pointed," "parietal placenta"). Then with the further aid of Coulter's *Botany of Western Texas* I proceeded to determine many species and to press them in amateurish fashion, but well enough to have them accepted by the University Herbarium.

At this time I also succumbed to another hobby, photography. I had a Kodak box camera of universal focus and I took a lot of pictures, developing the plates at night and printing the pictures on "Solio" paper in direct sunlight. Many of these I hardly surpassed later with a fine Leica camera; the negatives and plates are still in perfect condition after 60 years. My somewhat crude skill came in good stead in my researches later. Photography is a useful art, one which I recommend to all young scientists. It would save time, headaches and money, however, to get started under the tutelage of an expert.

Our country homes were heated with wood stoves, the kitchen being the center of attraction. For the winter's wood supply we had to drive to the woods about Round Rock in the heat of summer and chop the necessary supply. This is a region in which bat caves abound and I saw for the first time clouds of bats emerge from a cave exit like smoke from a smoke stack. I was to visit these caves frequently in later years. The local community came to these woods for posts with which to build our own telephone line. We all cooperated in this project, setting the poles and stringing the wire. I must have dug 30 postholes myself! Among the first messages that came over the wire was news that the Colorado River Dam at Austin had burst. I at once saddled my horse and rode through the mud, knee-deep in places, to the city. With my box camera I took pictures which are now of some historical interest, since with the series of new dams up the river such a flood is not likely to occur again.

In the spring of the same year I decided to visit the University to consult with Dr. William Bray, Professor of Botany, concerning the purchase of a microscope. His advice was: "Don't; come instead to the University and we will lend you a microscope free." It was good advice and I took it. The office in which I was interviewed by Professor Bray I later occupied as professor myself. The room suited me in spite of the odor of bats that occupied the spaces in the window frames. When I wanted a supply of bats, all I had to do was roll my office chair over to the window and, holding a net over the exit, capture all the bats I needed at the time. Studies of bat embryology and habits occupied several of my students. Few papers resulted from the extensive embryological collection, however, since, as will be described later, my attention was largely absorbed by the opossum.

I must digress here to relate my activity during the summer preceding my enrollment in the University in the fall of 1900. In a preponderantly German-speaking farm community southeast of Austin there were many immigrants who wanted their children to learn to read and write their native language and at the

same time to learn Luther's Catechism. I rode out into the community and drummed up pupils for a two-month's summer school—at \$10 per pupil (in advance). School "kept" from 7:00 to 12:00 a.m., and pupils rode as far as seven miles to the school. I made no extra charge for picking out tunes on the reed organ to accompany the singing of Sunday School and other German songs for the children. I had been raised on these songs and I still know many of them "by heart."

A more profitable phase of that summer's activities was my pursuit of Latin. I wrote out in English four books of the Aeneid, wrote out in Latin all of the exercises in Collar and Daniel's *Prose Composition*. Professor Penick refused to wade through these documents, but he made a bargain with me: if I made an A in the next advanced course in Latin, he would give me college credit for my summer's work. This I did, thanks in part to the basic training under the Wartburg professors, and in part to the inspiring teaching of Professors Penick and Fay.

After two years I secured the B.A. degree, still majoring in languages, and two years later I received an M.A. in Zoology and German. I "worked my way through" the University, but it was easier then than now. One year I was a student assistant; the last year I had the next higher rank, tutor. Earning one's expenses while going to school was, and is now, at the expense of social and recreational activities. However, I did keep my church affiliation, served as Sunday School superintendent, and for several years conducted church services once a month while the minister took his turn for a morning service in a country congregation. At that time all of the services were still conducted in German.

I suppose that the making of a scientist depends more on the influence of some one teacher than on any other factor. In my case it certainly was William Morton Wheeler, Professor of Zoology at the University of Texas during 1899–1902. He was ably assisted by Augusta Rucker who later took a degree in medicine at the Johns Hopkins University and then began practicing medicine in New York City. Perhaps it was Dr. Wheeler's reputation among the students which induced me to register for Zoology immediately upon my arrival. The move was a lucky break for me.

Dr. Wheeler was considered by many to be the most brilliant biologist in the country. He was also a great linguist, a voluminous reader in *belles lettres* as well as science, particularly the philosophical aspects of the subject. He was a close friend of Bergsson whom he visited occasionally in Paris.

Wheeler's lectures were fascinating. I took his entomology course and was permitted to accompany him on tramps in the field in those years when he was writing his famous "*Ants*." I like to claim that I helped him write the book by carrying his vasculum and eating his milk chocolate! I did, however, earn the chocolate by making some photographs for the book and by helping with the digging into ant burrows, notably the extensive nests of the leaf-cutting *Atta fervens*. Dr. Wheeler was also somewhat of an ecologist, and the rich fauna of the Austin region was spread out before me with considerable system and order. The distribution of plants and animals was interpreted for me by a master with all of nature as a laboratory. For example, it was apparent that the Carolinian fauna of the Atlantic and Gulf region followed up the flood plains of rivers, inter-

digitating with the Sonoran or Western fauna of the uplands between river valleys. A stone's throw would make a difference in the habitat of the agricultural ant, *Pogonomyrmex barbatus*, and the fungus-growing *Trachymyrmex*.

Dr. Wheeler left Texas to accept the post of Curator of Insects at the American Museum of Natural History in New York, inviting me to go with him. When he soon thereafter went to head the Bussy Institute of Harvard he again offered me an assistantship, which I also refused. My brilliant fellow student, Charles Brues, accepted and later succeeded his mentor as Professor of Economic Entomology at Harvard. Brues' boon companion, A. L. Melander, became a prominent entomologist out on the west coast. Other graduate students who came under Wheeler's influence in Texas at the turn of the century were: Walter Hunter, the psychologist; the gynecologist Harvey Matthews; and Jesse McClendon, the biochemist. As for myself, I applied at Columbia University for a fellowship in Germanic Languages and was, fortunately I think, turned down.

Dr. Wheeler was at his best with graduate students; he was bored with undergraduates. Witness the following three instructions I received in the summer of 1901 from his summer haunts at the Woods Hole Marine Biological Station. The diction is mine.

June 15. "For the fall class in entomology lay in a supply of well-fixed hellgramites and cockroaches." Both were easy to secure; the former abounded under rocks in the river and were easy to get at low water; the haunts of the latter I knew very well for I had previously worked in a local bakery.

July 15. "As I shall be very busy, I want you to take over the laboratory in Entomology."

August 15. "I will be too busy to fuss with the Entomology course at all; you are to take full charge of the course." That was my initiation in the teaching of science! The experience in entomology brought me an appointment for the summer of 1904 as Field Entomologist to study the cotton boll worm. Unfortunately, a bout with malarial fever prevented me from getting this desirable experience.

As someone perhaps truthfully put it, when Dr. Wheeler was through with Texas ants, he was through with Texas. He was succeeded by another great Zoologist, Dr. Thomas H. Montgomery, an authority on sex chromosomes. (Noting that the X-chromosome was superior to the Y-chromosome, he wrote an interesting paper on "*The Superiority of the Female Sex*"). He remained at the University for only a few years, and was succeeded by H. H. Newman, an authority on twinning, who came in 1908 bringing Dr. John T. Patterson with him.

As I had made little progress in one year on the subject I had selected for my master's thesis, the embryology of the bat, I decided to spend the summer of 1903 studying solitary wasps which I had noticed frequently on my tramps through the woods with Professor Wheeler. In two months of roaming along the sandy, open post-oak woods on the banks of the Colorado River, I filled a dozen notebooks with my observations and experiments and made many photographs. Result: my Master's Thesis on "*The Habits of Some Solitary Wasps of Texas*"; it was later published as a Bulletin of the University of Texas, and I occasionally see it listed by dealers in old books even today, 50 years after publication. I still

have solitary wasps as my hobby, making snapshots and motion pictures and publishing papers for technical and popular journals.

Dr. Newman returned to the University of Chicago in 1911, from which time on Pat guided the destiny of the Zoology Department, bringing it to the high status which it has now enjoyed for many years. Under Wheeler and Montgomery the department was a one-man show; Pat, on the contrary, built up a well-rounded departmental staff of which the University may well be proud. Not only was he later elected to the National Academy of Sciences, but three of the men whom he added to his staff have similarly been honored.

As I was about to obtain the Master's Degree under Montgomery in 1904, I applied for and was accepted as a research fellow to Dr. Edwin Conklin at the University of Pennsylvania. This was the year that our late colleague and friend, Dana Casteel, finished the work for the doctorate under Conklin. After reflecting on the condition of my eyes, I decided against accepting this \$750 fellowship, one of the best in the country. Professor Montgomery was furious, and told me so in unmistakable terms. This was another turning point in my career—away from science. While in general I was attracted to scientific investigation and felt that I had had some success as a teacher, I had no set goal; I was still drifting.

This time I entered politics. I ran for the office of County Superintendent of Public Instruction in Travis County and, fortunately or unfortunately, was elected—three times in fact, the last two times without opposition. For the period of my service I traveled, mostly on horseback, over the county's 1037 square miles, visiting its 170 schools, several of which were 40 miles away in the hills. In this day of anti-segregation I recall with some satisfaction that I was the first to pay some attention to the Negro schools of the county. I attended Negro community gatherings, held Negro Teacher's Institutes the same as for the whites. I was told that this was quite an innovation. That the people approved was demonstrated by my being twice re-elected.

What about Science during the five years of my running over the county?

There was much of interest in my environment. Travis County, Texas, is one of the most interesting areas imaginable; its geology determines the fertility of the soil and thus the economics as well as the ethnology of the population. A gigantic fault line, a thousand miles long, divides the county into two vastly different parts: a fertile two-fifths of black land to the east of the line, where thrift and prosperity are evident in the large painted houses and the big red barns; and a cedar-clad western three-fifths consisting of limestone hills among which the Colorado River meanders in great bends.

The hill country is sparsely inhabited. In conversation with some old-timers I learned what ecologists had overlooked—that before the days of barbed wire, when the Indians used to burn off the grass of the prairie, these hills were covered with a luxuriant growth of grass. When the salutary annual burning no longer kept down the cedar trees, these sprang up and choked out the grass, and the rains washed the top soil into the Gulf of Mexico.

Cedar and liveoak are the predominant trees in this section. The question once came up at the University as to the rate of growth in these two species. This was determined in the following manner. In looking over back numbers of the *Austin Statesman* while collecting material for my book, "*The History of County Super-*

vision in Texas," I learned that the Capitol of Texas was built of limestone. This splendid structure, contrary to popular belief, is merely veneered with granite, in response to the insistence of the owners of Granite Mountain at Llano. The limestone was quarried at Oak Hill to which the contractor built a railroad. Any trees now standing in the railroad right-of-way, I reasoned, must have grown up since the railroad was abandoned in 1886. So about 1923 a group of us (H. J. Muller, George Finlay Simmons and I and our wives) held a picnic on the old right-of-way, sawed down two of the largest trees of both species and counted the tree rings. Result: cedar and liveoak grow at about the same rate, in 35 to 40 years to a diameter of about 12 inches, if I remember correctly.

After resigning as County Superintendent of Schools I was fortunate in receiving an appointment as Professor of Biology at the Sam Houston State Normal Institute, now the Sam Houston State Teachers College, the oldest institution of college level in the state. Again I thought that this would be my life work. Nowhere have I encountered deeper and more sincere devotion to teaching and more earnestness on the part of the student as at Huntsville. In retrospect I feel that the three-year period spent there was among the most satisfactory of my life. But the question before me at this point is: did these three years of science teaching and study lead me closer to a scientific career?

In spite of having to teach thirty classes a week and to serve on several faculty committees, I am able to recall much interesting study on my part, especially in Botany in which I was poorly prepared. Every pleasant Saturday found me out in the piney woods botanizing. I knew where to find illustrative material for the class: *Marchantia*, fruiting mosses, and fern prothallia. In small lakes and ponds I found *Chara* and *Nitella*, the latter, along with stamen hairs of *Tradescantia*, useful in observing the flowing of protoplasm. I also learned to induce conjugation in *Spirogyra* when wanted. In one lake, I am now convinced, I discovered *Chaos chaos*, the giant ameba, which I later saw in considerable numbers in Dr. Kudo's laboratory in Illinois. However, at the time I made nothing of the discovery because a well-known protozoologist insisted that what I had seen was not an ameba but a slime mold. But, slime mold or ameba, my students were enthralled at the sight, as I was also.

Having had some experience with systematic botany of the flowering plants, even though this was self-taught, I prepared and published in the Bulletin of the University of Texas (1913) a "*List of the Trees and Shrubs Occurring in the Vicinity of Huntsville, Texas*," with notes on the ecology of the region. Solitary wasps abounded everywhere and my old friends were not passed by unnoticed. I was the first to record the manner in which *Eumenes belfragei* builds and "decorates" her beautifully symmetrical jug-shaped nest (*Animal Behavior*, Vol. I); I sent two undescribed species of ants to Dr. Wheeler, with ecological notes—these species, which raise fungus gardens on caterpillar droppings, were *Atta hartmanni* and *Trachymyrmex caroli*.

The various materials that I gathered on my forays were shared with my students. I was learning along with them. I feel that, while I was too immature scientifically to impart profound generalizations, I was able to awaken a warm interest in the study of plant and animal life. I have ever since held that engendering enthusiasm is more important than handing out facts, and I have found

some graduate assistants better at this than some permanent members of the staff. I have myself taught things that afterward turned out to be wrong. What of it? The wide-awake student will find out these mistakes for himself if he has been taught not to believe everything he reads or even hears from his professors.

Many years ago, long before the University building boom, Pat invited Professor Conklin to deliver a lecture on Evolution. This was delivered in the Methodist Church, the auditorium of the old main building having been declared unsafe—structurally unsafe, that is, not “unsafe” for a lecture on evolution. This was before the Scopes trial! On this occasion Pat asked me to show Conklin a live tarantula out in nature, and we had no trouble doing so. Facetiously, the professor asked if we had “planted” the spider for his special benefit. I also had the pleasure of piloting Dr. H. J. Comstock of Cornell around among the hills and into the bat caves. In one cave he found a louse which proved to be a new genus, and which he promised to name after me!

A chance remark by my father at about this time probably changed my life's work. Noting that I made up the entire biological faculty of the Huntsville college, he remarked, “Now you are the one-eyed king among the blind,” quoting a German expression. I got the point, and thus after three years at the Sam Houston Institute I decided to accept Professor Patterson's offer of an instructorship in his department. My motive in studying at a large center of learning was genuine; I recall that at Huntsville my salary was \$2500 (good for that day) and I left it to accept a job at less than half that. Besides, at Huntsville I had a cow, 300 chickens, a large garden and plenty of credit at the grocery store in exchange for produce! What a bonanza to leave behind!

This discrepancy was, however, cancelled out by a streak of good fortune following a burst of unusual hard work. The last year at Huntsville, Dr. Louis Bibb, a young Austin physician, and I wrote two books for Texas schools: the *First Book of Health* for the third grade, and *The Human Body and Its Enemies* for the fifth grade. In September we defeated all of our “Yankee” competitors by securing the state adoption. How could we lose? Louis Bibb was secretary of the State Medical Society and I had made a reputation as an “Educator.” Besides, did we not mention Texas a hundred times in the books? Our first year's royalties amounted to \$7,000 for each of us, truly a magnificent sum.

I was now embarking, with Pat's encouragement, on some researches that were to occupy my waking hours for the rest of my life. I had at last, thanks to my new environment, found my calling, and the road before me was clearly outlined. Pat's offer to join his department included one “perquisite of office”: the privilege of studying the embryology of the opossum—at my own expense! At that time the department was poor and there were no facilities in 1913–1915 for keeping animals on the campus. Even when the opossum collecting season was at its height in February and March, 1917, when we collected several thousand eggs and embryos, we had only primitive facilities—wooden cages built by myself under one end of an old army barracks which was used for laboratory instruction before the discovery of oil on University land. In 1923, however, Pat secured legislative appropriation for a new Biology building.

On my arrival in Austin I had found Pat engaged in what to me were sensationally interesting research projects. It was in his laboratory that I had the thrill

of seeing my first mammalian embryonic vesicle in the open uterus of the armadillo. Even after handling several thousand vesicles of the opossum and more than a hundred from the monkey I still recall the impression which that first armadillo vesicle made on me.

A further most interesting series of observations to which Pat introduced me involved the life history of *Paracopidosomopsis*, the tiny wasp parasite with the big name. This study was but an expansion of Pat's interest in polyembryony which the wasp exhibits in an astounding manner, for a thousand offspring often arise from a single egg. The wasplet is a potent parasite of the cabbage caterpillar. These and other basic studies which greeted me in the Department of Zoology were eye-openers and inspiring activities. I did not myself "go to the ant, thou sluggard" as Professor Wheeler would have ordered, but, following Pat's example, I selected the opossum for my researches for the next twelve years.

Why the opossum? For the same reason that Pat selected the armadillo: the prairie and the creek bottoms were full of both species and nobody before us had yet bothered to study the development of these, the most bizarre of all American mammals. It was at the University of Texas that the essential characteristics of their mode of reproduction were cleared up.

For several years, with the aid of students (notably Kenneth Cuyler, now at Duke University, and George Hamlett, now at Louisiana Medical School) I hunted the animals in the field. I also took care of them, operated on them, secured, fixed, sectioned the eggs, stained and photographed the sections myself. The source of light for photomicrophotography was a home-made illuminator: a bull's-eye lens set into a tin can which slipped easily into a slightly larger can for focusing, the light bulb being in the upper can. I have a little essay in the bottom of my barrel entitled "In Praise of Poverty." I realize, of course, that the poverty situation can be overdone, but I wonder if your young graduate students don't have too much done for them at the large, well-equipped centers. After all, they will likely get jobs at small colleges where they will be more or less on their own.

As our method of procedure in collecting embryonic material required surgery, a score of pre-medical students volunteered their help, working far into the night during the height of the collecting season. There was no luxurious operating room available; we worked in the physiology laboratory when it was not otherwise in use. Thinking back on the enthusiasm which our young helpers exhibited 40 years ago, I would suggest that research laboratories be not far removed from the teaching laboratories. It is good even for Freshmen to sense that there is something new, even mysterious, going on around them. They will snoop to get an idea of what it is and if the researching professor will stop and explain the matter he may be making research scientists out of some of the choice pupils under his influence.

When Pat assigned me to develop physiology in the department I again felt like that "one-eyed king among the blind," since I knew only too little about the subject. Using my book royalties I spent four summer quarters at the University of Chicago, studying physiology under the great A. J. Carlson, neurology under two masters, Herrick and Coghill, and biochemistry under Fred Koch. These studies were supplemented at Columbia University in the summer of 1917 by

clinical pathology and clinical bacteriology, the latter under the famous Hans Zinsser. A year's leave of absence (1917–1918) was spent at the Wistar Institute and the University of Pennsylvania, which enabled me to make rapid progress in working up the rich collection of eggs so that I could complete a monograph on the subject in 1919.

In the ten years following 1915 I published several papers on the early embryology of the opossum, passing gradually to a consideration of the physiology of reproduction. Later I studied the embryology of the monkey but again I relegated the embryology to second place, preferring to pursue physiological problems.

To the legitimate question: of what use are those opossum studies, I have a ready answer. Not, however, that their possible applicability ever constituted a motive on my part. Knowledge of physiological processes carries over from one species to another, including man. Some specific examples may be given. (1) The newborn opossum, a mere embryo, has been used by Burns to solve problems of sex reversal and the genesis of hermaphroditism. (2) The blood of the newborn opossum contains globulin but no plasma cells, the supposed origin of globulin according to the books. There are likewise no small lymphocytes, the supposed origin of fibroblasts in inflammation; inflammation can be produced in the newborn opossum, however. Therefore, according to Block, hematological theory may require basic revision. (3) It was on the opossum material that Painter discovered the X-Y condition in spermatogenesis, later proving this for man also. (4) The opossum taught me two basic principles, now generally accepted, which have had a great influence on gynecological thought: I clinched the principle of the short functional life of the unfertilized egg, and the unexpectedly rapid ascent of the spermatozoa in the female genital tract. I had to demonstrate the latter by rather spectacular experiments in the rat; now, after 30 years, the subject is receiving serious attention from gynecologists as I have shown in my recent paper, "*How do sperms get into the uterus?*" (1957. *Fert. & Steril.* 8:403–437).

These examples serve to illustrate the fact that basic studies sooner or later find a practical application. I never bothered to ask: is there any use in this study? The main thing was that I was satisfying my own curiosity and having fun doing the experiments.

The work on the opossum proved to be a preliminary training for my studies on the monkey, for in 1925 I wrote up, with Pat's blessing, a prospectus for an expedition to the Philippine Islands to gather monkey embryos since I felt that such a study would constitute a necessary preliminary to the securing of comparable human stages. With the aid of Arthur LeFevre, formerly a Professor of Latin at the University of Texas and an intimate friend of Will and Mike Hogg, members of the board of the Texas Oil Company, these loyal University alumni were induced to provide the larger portion of the expenses for the proposed two-year "Texas" expedition. The National Research Council also made a grant to the amount of five digits. I sent my proposal to Dr. George Streeter, Director of the Department of Embryology of the Carnegie Institution of Washington, whose laboratory was a part of the Johns Hopkins Medical School in Baltimore. Dr. Streeter's reaction was an invitation to me to join his laboratory and study monkey embryology the "safe and sane way," bringing the monkeys to the labo-

ratory and thereby avoiding the dangers of tropical infections for myself and family. George Corner had already demonstrated that the *Rhesus* monkey made a good laboratory animal once one learned how to house and handle them. So the "Texas" expedition, and my trip around the world, went by the board and in 1925 I resigned and joined the Carnegie laboratory. Sixteen years of research in primate embryology and reproductive physiology ended after I had secured over a hundred monkey eggs and embryos, completing the story of the first thirty days of monkey development. This was published in a *de luxe* edition in the *Contributions to Embryology* by Heuser and Streeter, and in which my name was omitted from the title by the editor, perhaps unwittingly. I was myself more concerned with the physiology of the female monkey, contributing my share to the unraveling of the cause of menstruation and the sequence of events in the menstrual cycle, especially the time of ovulation. These studies, supplementing those of P. E. Smith, George Corner, George Bartelmez and others have had a profound effect on gynecological thought the world over.

As I saw my obligatory retirement from the Carnegie Institution approaching, I decided to accept a six-year appointment as head of the Departments of Zoology and Physiology in the University of Illinois. These were the war years. Again I retired, and this time, having a broad knowledge of reproductive physiology, I accepted a position with the Ortho Research Foundation at Raritan, New Jersey, becoming Director Emeritus in 1958.

The Department of Zoology of the University of Texas, under Pat's versatile direction, has of late years become a renowned center for research, with emphasis on genetics. Pat's interest in genetics began many years ago with his modest but thorough course in the subject (and which my wife Eva took, and made an A!). Genetics was given a boost by his bringing in T. S. Painter and H. J. Muller. I took Muller's lectures myself and, incidentally now that he has attained the Nobel Prize, I am not a little puffed up by recalling that I urged him to lose no time in studying the effects of x-rays on the mutation rate of *Drosophila*. To my mind Mavor, at Union College, had at least demonstrated that something significant lay in that direction. Muller's flies were radiated by my friend, Dr. Richardson, an accommodating local radiologist with a feeling for basic research. This proved a lucky break for Muller and for the Department, for it opened up a new approach to Genetics. I am happy to have had a small part in the first successful study.

In view of Pat's consuming interest in the genetics, physiology, and ecology of *Drosophila* one is apt to forget his contributions to embryology, his first interest. In my day I always thought that two basic courses of the department were the most outstanding: beginning Zoology, conducted by the fine scholar and gentleman, Dana Casteel, and the course in embryology given by Pat. Each student in the embryology class was equipped with both compound microscope and the newly invented and improved binocular stereomagnifier, the former for studying the slides, the latter to aid in the dissection, with needles and fine-pointed forceps, of the 12 mm. pig embryo. Pat always maintained a high standard for his students. There was no pampering; his recommendations for admittance to medical schools meant a lot to the Deans of the top schools of the country.

To my young friends who happen to read this condensed sketch of nearly 60

years of teaching and research I would say that hard work and perseverance always bring results. Consistent prosecution of well-planned researches may, with meditation, lead to generalizations which are the key to progress in science. I used the following quotation as my motto in my 1932 monograph on the reproduction of the *Rhesus* monkey: "It is certainly not the obligation of science to explain everything. We must collect facts. Once facts have been assembled, the explanation emerges of itself." It doesn't often happen that a natural law looms up in the serendipity of the scientist, but it can happen to any consistent researcher who stays on the job. Certainly it does not happen without a background of a multitude of personal observations. To acquire such a background takes hard work, often long hours, the productive use of long academic vacations, and "guts." The IQ of a genius is, fortunately for most of us, not essential to making substantial contributions to knowledge and the satisfaction of having made them.

I do not recommend my own devious course toward a scientific career; instead I recommend acting upon the spirit of the saying, "The world stands aside for the man who knows where he is going." It is best to make up one's mind when young and get going toward a goal—not omitting those amenities of the well-rounded life that make for a whole, completely social person which, I may add, describes almost every professor in my rather wide acquaintanceship.



Left to right: H. J. Muller, the author, G. H. Parker, and J. T. Patterson. Taken on the occasion of Dr. Parker's visit to Austin about 1923.

High Islands and Low¹

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This evening I am inviting you to travel westward, far westward, with me as a member of Task Force 7, under the auspices of the Atomic Energy Commission, to the Pacific Proving Grounds in the Marshall Islands and beyond. Our trip, and the special work we are doing, will take us at times far from the Proving Ground. We will not discuss the work, but by no interpretation of our orders is it forbidden that we reminisce about what we saw in the Southern Marshalls and the Eastern Carolines, now under the United States, administered through the Department of the Interior (I should think that this phase of operations should be designated the Department of the Exterior) as a part of the United Nations Trust Territory.

We are at the Hopkins Airport in Cleveland on a late July morning, 1956. We are scheduled to take this trip with three scientists from the Genetics Foundation at the University of Texas, and so our seeming diversion to the Lone Star State makes some sense.

Fasten your seat belts. No smoking.

We will see that sign flash forty times before we set down again at Hopkins Airport on September 4th. Cleveland, Chicago, Dallas, Austin, San Francisco commercial airport, Travis Air Force Base near San Francisco, Honolulu, Kwajalein, Eniwetok, Bikini Atoll, Eniwetok, Majuro Atoll, Kwajalein, Eniwetok, Ponape in the Carolines, Eniwetok, Kwajalein, Honolulu, Travis Air Force Base, San Francisco, Chicago, Cleveland—forty take-offs and landings. You only counted thirty-eight. But you forgot those two runs on the Hickam airstrip in Honolulu several hours apart and then the orders back to bed and up the next morning for another try. No wonder MATS (Military Air Transport Service) has such a fantastic safety record. If all four of the big engines aren't just right you don't take off for Kwajalein over 2300 miles of water until they are. Of course we haven't counted the trips island hopping in a helicopter, where you make a round-trip tour of the atoll and land on perhaps half a dozen islands between 9:00 A.M. and noon.

We will travel on the two-motored planes of Braniff, and the big four-motored transcontinental planes with good-looking hostesses and good food, on the big MATS planes across the Pacific, each one capable of carrying sixty tons of cargo but now carrying mostly military personnel, sergeants instead of pretty hostesses, cold box lunches instead of hot meals, and liquid ration of coffee and water only. We will fly to Ponape by sea-plane and land on the lagoon, and take off with JATO (rockets which blast the plane off the water). And in the islands we'll travel by fast water-taxi from one small island to the next; by LST, an ocean-going vessel over a hundred yards long, without a keel and capable of landing at an open beach, between Bikini, Rongelap, Rongerik and Eniwetok Atolls, a

¹ This is a modified version of a talk delivered to the Century Club of Wooster, Ohio, in 1956.

sea cruise of a few hundred miles; by Duck (an amphibious craft) off the LST to certain islands and over the islands in this strange mechanical monster, equally at home powering its way over the lagoon, rolling up the beach between coral heads, or plowing through the jungle, wreaking havoc on unsuspecting palm trees in its path. We will sometimes go by LCM boat, generally referred to as an M-boat, several miles from one island of the atoll to another. And if you wish you may take a trip in an outrigger as some of my friends did, and as I regretted not doing.

On land we will be travelling by truck, by jeep, or on foot, but not likely by car. By these several and sundry means we'll travel some 20,000 miles in less than six weeks. But more significantly, perhaps, we might describe it as "flying through history." For we will have flown from the great centers of western civilization to the sites of ancient primitive cultures, and quite possibly we will have visited the symbolic center of the final quotation mark after that human autobiography which we call history.

In 1846 Wiley and Putnam published Herman Melville's *Typee*, with the subtitle "A Peep at Polynesian Life during a Four Month's Residence in a Valley of the Marquesas." This served as a sizable part of my literary fare on the trip out from San Francisco to Honolulu and on to Kwajalein in the Marshalls. I recommend it as required reading for anyone making a trip to the Pacific Islands. Over a hundred years later it can still serve as a guide to much of what will be seen in native villages today, and of course it has the style and charm of the master craftsman.

The first paragraph of *Typee* presents a soliloquy on the hardships and rigors of a six-months cruise on a whaling vessel to reach the Marquesas Islands and ends in these words: "Oh! ye state-room sailors, who make so much ado about a fourteen days' passage across the Atlantic; who so pathetically relate the privations and hardships of the sea, where, after a day of breakfasting, lunching, dining off five courses, chatting, playing whist, and drinking champagne punch, it was your hard lot to be shut up in little cabinets of mahogany and maple, and sleep for ten hours, with nothing to disturb you but 'those good-for-nothing tars, shouting and tramping overhead,'—what would ye say to our six months out of sight of land?"

As I read that paragraph I couldn't help wondering what Melville would have thought and written of those air travelers of today who grumble about slight inconveniences as they travel in hours literally the distances covered by those mid-nineteenth century travelers in days. We left Kwajalein on Sunday evening, September 2nd, at 6:30 P.M. Cleveland time and were in Cleveland Tuesday morning, September 4th, at 10:00 A.M., a distance of approximately 7500 miles, and this included several hours on the ground in Honolulu, a seventy-mile taxi ride between the two airports in the environs of San Francisco with several hours wait there, and a couple of hours in Chicago. And again Melville came to mind when, at the Officers Club on Eniwetok, the evening before we left for home, an air force captain told of recently flying a jet the 2500 miles to Honolulu in less than four hours. More than once I thought of Melville and his valley of Typee in the Marquesas Islands of 1850 during our visits to the Kapingamarangi Village on the island of Ponape in the Eastern Carolines in the summer of 1956. Much

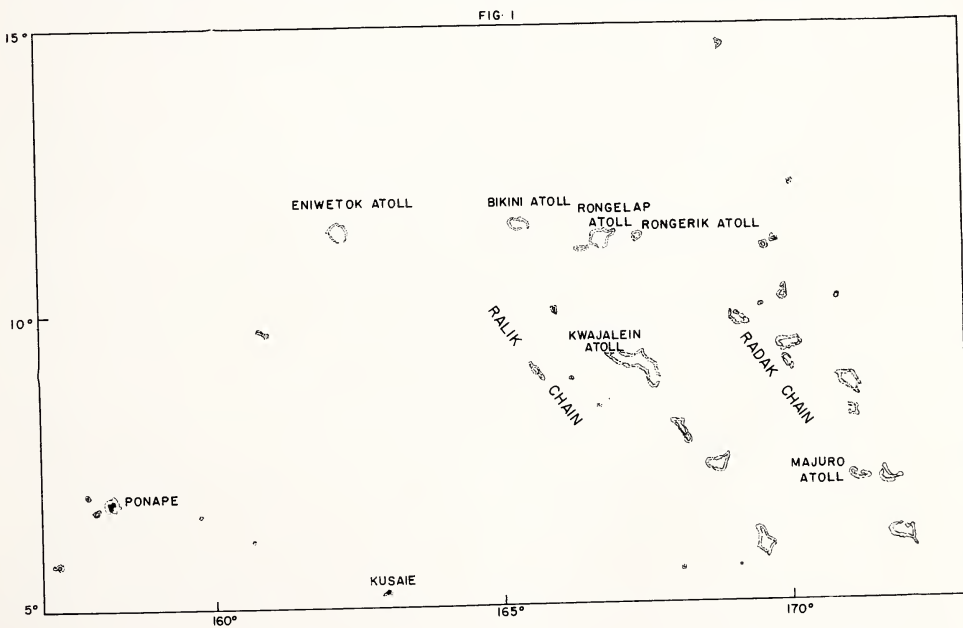
that we saw there could have come right out of the pages of Melville, and for that matter Typee might just as well have been written by Captain Cook in the late 18th century or by some bold mariner had he chanced upon the islands a thousand years earlier.

Between latitudes 30 North and 30 South, the eastern two-fifths of the broad Pacific is almost devoid of islands except for the Galapagos group off the coast of northern South America. But a look at a good map will show that the western three-fifths of the Pacific between these latitudes is liberally sprinkled with thousands of islands. These may be roughly divided by two lines running north-west-southeast into three great island groups, an eastern Polynesian group, a middle Micronesian group, and a western Melanesian group.

Polynesia includes the Hawaiian Islands, the Line Islands including Christmas Island directly to the south of Hawaii, the Marquesas, the Society Islands, of which the most famous is Tahiti, and the Tuamotu group lying between the Marquesas and Society Islands but stretching further to the south and east to Pitcairn.

Melanesia forms a chain of island groups in order from northwest to southeast: New Guinea and New Britain, the Solomons, New Hebrides, and the Fijis.

Micronesia (Fig. 1) lies roughly between Melanesia and Polynesia, stretching from approximately latitude 20 North, longitude 140 West to latitude 10 South



on the international date line, that is from northwest to southeast. While most of the islands of Polynesia and Melanesia are small, the Micronesian islands are still smaller. To the north lie the Marianas, a north-south chain about six hundred miles in length and including Guam, Rota and Saipan. South of the Marianas are the Carolines, stretching for two thousand miles in an east-west direction. In the Palau group of the western Carolines is found Babelthuap Island, approximately 150 square miles in area and the largest of the Micro-

nesian islands (but less than a fourth the size of Wayne County, Ohio). Yap is another of the larger islands in the western Carolines. In the eastern Carolines the largest islands are Truk, Kusaie and Ponape, the latter being the second largest island in Micronesia with an area of approximately 100 square miles. A few hundred miles to the east lie the Marshalls, in about the same latitude. South and a little east of the Marshalls are the Gilberts, close to the equator, and still further south and east lie the Ellice Islands.

In his *"The Fortress Islands of the Pacific"* published in 1945 William H. Hobbs gives an intriguing account of the geology and geography of the islands of Pacific Oceania and of their value as military bases. In brief his thesis is as follows. The islands of western Oceania, Melanesia, the Aleutians, the Marianas, and the western Carolines are what he terms arcuate islands formed by crescentic folds and upthrusts from the floor of the Pacific. Such folds form mountain ranges rising from the Pacific floor with their peaks forming a chain of islands. The folds tend to be steep on their eastern face and with gentle slopes on the western face. Eastward of each fold lies a deep trough in the sea. Thus the north-south chain of the Marianas may be taken as an example with the Nero Deep just to the east almost six miles in depth, the second deepest sea trough in the world. East of these arcuate chains lie what he terms the strewn islands, the islands of most of Micronesia and all of Polynesia. These are not mountain chains, but rather have been formed by groups of volcanoes at one time active on the sea floor, eventually rising above the surface. Some of these have become inactive, eroded, and formed atolls, rings of coral island motus or islets from the reef originally surrounding the volcano, now eroded and submerged. He recognizes several types of island groups forming transitions between these stages, the volcanic island, with its surrounding coral reefs; the almost-atoll, where parts of the crater still stick above the surface in the lagoon surrounded by coral reef and developing coral islands; the typical atoll, with coral reef and islets surrounding a lagoon, and still later stages in island evolution, partly raised atolls with shallow lagoon, and raised atolls with the lagoon repaced by flat-topped phosphate rock. It will be seen that Hobbs accepts the Darwin-Dana theory of the origin of atolls.

However they were formed geologically it would be obvious to even the most casual observer traveling by boat or plane through Polynesia and western Micronesia that the islands are of two very distinct types, a relatively few larger mountainous islands and a myriad of tiny islets, the latter typically arranged in circles, quadrangles, or less even geometric figures, but generally each island forming a long narrow strip, obviously arranged like long narrow beads on an encircling coral reef surrounding a lagoon. This is what we mean by high islands and low.

In 1896 Robert Louis Stevenson published a treatise entitled *"In the South Seas"* and here he describes vividly this contrast as follows:

No distinction is so continually dwelt upon in South Sea talk as that between the "low" and the "high" island, and there is none more broadly marked in nature. . . . On the one hand, and chiefly in groups of from eight to a dozen, volcanic islands rise above the sea, . . .

their tops are often obscured in cloud, they are all clothed with various forests, all abound in food, and are all remarkable for picturesque and solemn scenery. On the other hand, we have the atoll; . . . rudely annular in shape; rarely extending beyond a quarter of a mile at its chief width; often rising at its highest point to less than the stature of a man—man himself, the rat and the land crab, its chief inhabitants; not more variously supplied with plants; and offering to the eye, even when perfect, only a ring of glittering beach and verdant foliage, enclosing and enclosed by the blue sea.

The Hawaiian Islands, which some of you have visited, are of course "high islands" of Polynesia. Our party spent several days on Oahu in the summers of '55 and '56. Through the hospitality of members of the Zoology faculty at the University and of Dr. Mitchell of the U. S. D. A. we saw a good deal of the island, downtown Honolulu, Waikiki Beach and its fine hotels with their gardens, and curio shops. We drove out past Pearl Harbor and Scofield Barracks, past sugar cane and pineapple plantations, over the Pali drive up through the mountains and along the beach past Diamond Head. We made a tour of the University and of the famous Bishop Museum, and took a hike on a mountain trail along the shoulder of Mt. Tantalus far above the city of Honolulu. My most vivid impressions of Oahu: the air-conditioned climate, almost every home surrounded by gardens with a rainbow array of flowering trees, shrubs and herbs including orchids; steep serrated cliffs in the mountains, not bare rock but covered by dark green verdure; the polyglot of racial types seen on the streets and all talking good mid-Western American; the irony of Hawaii not having been given statehood long ago. But Hawaii would serve as subject for several papers. Let us go for examples of low and high islands to the Marshalls and Eastern Carolines 2500 miles southwest of Honolulu.

We shall take the Marshalls in general as examples of low islands and the Majuro and Rongelap atolls in these islands for more specific reference as both lie outside the Pacific Proving Grounds test area, the former in the southern Marshalls and the latter in the northern Marshalls. We shall visit the island of Ponape in the Eastern Carolines as an example of a high island.

In "*Fortress Islands of the Pacific*" Hobbs introduces the Marshalls as follows: "This double chain of atolls lies to the northwest of the Gilberts and is separated from them by three degrees of latitude. Like the Gilberts, the Marshalls trend northwestward with their eastern chain known as the Radak (Sunrise) chain, and the western as the Ralik (Sunset) chain. These chains contain thirty-six larger atolls with 876 motus (islets) and reefs which stretch from Ebon at the south in latitude 4 degrees, 34 minutes to Eniwetok at the extreme northwest in latitude 11 degrees, 30 minutes North."

These almost nine hundred islets have a combined area of a little less than 70 square miles, approximately one-eighth the area of Wayne County. Kwajalein atoll about midway in the western chain is the largest in land area with 6.33 square miles. It also has the largest lagoon area, about sixty miles long and with some 90 motus or islets. It is in fact one of the largest atolls known. We visited Eniwetok, Bikini, Rongelap and Rongerik atolls in the northern Marshalls and

lying in the western chain, Kwajalein (about midway between northern and southern Marshalls), and Majuro in the southern Marshalls and in the eastern chain. We were on approximately twenty different motus or islets in these atolls.

What does an atoll look like? This depends on the viewpoint, whether one is flying, say, at an altitude of 10,000 feet above it, coming in low for a landing or flying a few hundred feet up in a helicopter, approaching it by sea in an LST, riding over the lagoon in a small craft, or actually walking along the beach of a motu on the lagoon side or on the sea-side of the islet. This is what a typical atoll looked like to me from the window of a MATS plane flying at an elevation of 9,000 feet. It was as though a modern artist had taken a giant canvas and first laid down a background of dark blue with tiny ripple marks to represent the waves of the sea. Next he had taken a brush, dipped it in light brown paint and traced a rather regular quadrangular line. This line was the coral reef, but submerged. Next he had traced a white line just outside the brown, the breakers in the shallows off the reef. Outside the white line he had laid down a broad light green strip merging gradually into the dark blue, the shallow water on the seaward side of the reef. Finally he had daubed dark green splotches, some forty or fifty of them, some tiny circles, but mostly long strips of green and irregularly spaced but always directly on the light brown line of the reef. These were the islets covered with vegetation, mostly cocoanut palms, but these indistinguishable as such at that height. At the corners of the quadrangle the green splotches were somewhat larger and roughly shaped like a boomerang. For some reason the islets at the angles of a reef are generally, though not invariably, larger. One more item: a few daubs of dark brown paint interspersed with the green blotches along the light brown line; tiny islets sticking above the surface of the water, but with little or no vegetation, probably covered at high tide. Such is an atoll as I saw it from 9000 feet in the air and it ought to take a prize at a showing of modern art. But the canvas is large; the average atoll will be twenty or thirty miles from one end of the lagoon to the other. And on some of the atolls the light brown line will be interrupted at one or more points, a deep water passage into the lagoon, and in other atolls the islands will mostly be in a semi-circle on the eastward side of the lagoon. But I chose to describe a particular atoll which we flew over and I don't even know its name, or whether it had any.

From the beach on the lagoon side of an islet one can see adjacent islets in the circle both to the right and the left, but those across the lagoon twenty or thirty miles away are invisible as the islands themselves have a maximum elevation of less than fifteen feet generally, and the cocoanut palms are not likely to be more than forty or at most fifty feet tall. We shall return to the atoll islands for closer inspection later, but for contrast let's view a typical high island from the air.

On August 22nd our party of nine boarded an SA-16 Aircraft on the Eniwetok air strip, with Captain Stahley and crew of three in charge, bound for Ponape some seven hundred miles to the southwest in the Eastern Carolines. This was a sea-plane and several hours later we came in for a landing on the lagoon between the reefs and the main island. The view of Ponape as we came in for the landing (if that is what a sea-plane hitting the water is called) is one which I shall not forget. I'm not kidding myself; it will get fuzzy around the edges, but the main show will be there years hence along with my first view of Naples Harbor, the

Grand Canyon from the South Rim, the sweep of the Teton range in Wyoming, the unearthly shimmer of Death Valley in the late spring, the coloring of Bryce Canyon, the majesty of Yosemite valley, and the fall coloring on a valley road in Wayne or Holmes County in a good year. These are things you can't take from me and on which I pay no income tax. If I did I'd be broke.

What is it that makes a view worthwhile? I think it is partly a matter of contrast. If the trees here were always colored as they are in October we would probably all be color blind. And physical conditions may help. For one thing we had good windows for viewing in that navy plane, which is more than can be said for the tiny windows spaced well apart in the big MATS planes. Then on those two-engined planes used in flying in the islands we always had to wear a "May West" on take off and landing, and the landing sign flashed long before the landing. The May West is a horse collar affair weighing too much for comfort. Mae must have been a buxom lady in her day. But the navy plane was outfitted with a much more modest affair, worn the same way but better described as a Katherine Hepburn or a Grace Kelley, and so one could observe the scenery in some comfort. Then after looking at all those tiny atoll islets and tramping around over many of them I could hardly believe that out there in the wide Pacific, in that region at least, there could be anything like Ponape, a real chunk of land where you could undoubtedly get lost and lose the impression that there was a sea around. Without piling on the adjectives I'll just say soberly that my first glimpse of Ponape struck me as something out of this world or more properly something out of this sea. There it was and I could hardly believe it. You may smile when I tell you that it was an island only about thirteen miles in greatest diameter, but compared to those islets where you stand in the middle and see the sea a few hundred feet on one side and the lagoon a few hundred feet on the other side, it was something to wonder about. Perhaps it was a little of the same kind of exaltation which came to the ancient mariner on sighting land after a few months on the open sea.

As the plane came in from the north over Langor Harbor, circled and lost altitude one saw the main mass of the island, with mountain ranges, rather rugged, some of the peaks rising to around 2500 feet, dark green with luxurious tropical vegetation, this broken in spots with lighter green patches which as the plane approached closer were seen to be clumps of palms on the mountain sides, the tops of the peaks hidden in masses of white and gray rain clouds. deep valleys cutting in between mountain ranges, the shore line deeply cut by estuaries where the valleys and their streams came down to meet the sea; and on the west shore of the largest estuary the settlement of Colonia, with its metal roofs glinting in the brilliant sun, more of a town than we had seen since leaving Honolulu (I don't count military installations, however extensive, as towns). Encircling the main island a few miles off shore were the coral reefs, with their usual garnishings of white surf and light green shallows. Between the reef and the main island were several small islands, small replicas of the main islands, rugged, not flat, though of course the hills were also in miniature. On one of these was the landing ramp and concrete strip for the plane to taxi up and come to rest. To the west of the harbor a huge mass of rock with a sheer face jutted out of the sea. A few tiny craft could be seen on the surface of the harbor. This is a poor description of a

sight of surpassing beauty to a landlubber far from his native environment, mid-western U. S. A. A few moments later after careful reconnoitering (it is not healthy to set a sea plane down on a submerged coral head) the pilot feathered the engines and the plane settled down in the water at perhaps forty or fifty miles an hour, with the pontoon on the right wing swishing along in the water and that on the left wing a few feet above the surface, water swishing up against the sealed windows, and the plane rapidly losing speed. As we approached the landing ramp, a gentle concrete incline, the landing wheels were let down out of the sides of the plane and we taxied up and along a concrete strip, turned and came to a halt. We debarked and deposited our luggage in the picket boat which serves also as a crash boat for the sea-planes. While waiting the arrival of a Transoceania plane, flying weekly the rounds from Guam to Truk to Ponape to Majuro and back, we were greeted by friendly officialdom with a welcome to Ponape. In a half hour or so the other plane had arrived and with its passengers we took the half-hour ride across the lagoon to the dock at Colonia on Ponape. After a short wait on the dock, surrounded by a large group of natives, most bare-foot and in rather tattered garb, apparently on hand to see the weekly arrivals on the plane, we were transported (or should I say carted) in very ancient jeeps, with springs long since broken out of the seats, over rather a rough street, yes, a very rough street, up to the crew's quarters, a quonset hut with open sides below and thatching above, where we were to stay for the next eight days. The accommodations were adequate, semi-private rooms, flush toilet, hot shower, electricity and a water cooler which ran only lukewarm water. On the way, the galley, where we were to take our meals, was pointed out, the fare adequate though somewhat monotonous with rice served as a base every day twice a day without fail. But there were other dishes including some native stuff such as yams, breadfruit, and baked bananas.

Perhaps before we start the exploration of the town of Colonia and adjacent regions of Ponape Island it would be well to lay a very brief historical background. Most of the information was gleaned from a pamphlet issued to all visitors and prepared by Henry Hedges, District Administrator of the Ponape District of the Trust Territory of the Pacific Islands, and in conversation with the administrator, his wife and other officials.

Since the second world war the United States has acquired the administration of a Trust Territory under the United Nations which includes the Marshalls, Carolines, and Marianas. This territory is administered by the Department of the Interior with an annual operating budget of \$5,000,000 divided about evenly among the six districts. These six districts are: the Marshall Islands, the Marianas, and four in the Carolines, namely, Ponape and Truk in the Eastern Carolines and Yap and Palau in the Western Carolines. There are approximately 60,000 natives in the territory, about 12,000 in the Ponape District and 11,000 in the Marshalls. There is a general administrator of the Trust Territory and under him district administrators for each of the six districts. Each administrator has associated with him a group of twenty to thirty Americans, most of whom have their wives and families in residence on the site of operations. The personnel on Ponape is distributed among the following departments: administration, education, public health, agriculture, construction and maintenance, land

and claims office, weather bureau, and special projects, thirty-four members in all but that includes some of the wives. Much the same organization is to be found at the headquarters for the Marshall Islands District on Majuro Atoll. These people are paid comfortable salaries, of course scaled according to the position; they sign up for a two-year period with three months furlough in the States, travel expenses paid. On Ponape, where most of the personnel are young people in their early thirties the turn-over in personnel has been high, due among other things to the fact that some wives simply could not take the restrictions of life as it must be lived in such a place. There are inconveniences if not actual hardships. The average age of the personnel on the Marshalls was considerably older, and more of the people there were adapted through long experience to life on a tropical Pacific island. Of course there are many natives hired as helpers in the project, some few of them in responsible positions. Thus the administrator of education in the Marshalls is a native trained at the University of Hawaii in Honolulu. It is the business of the Trust Territory personnel to work with and for the natives on these islands to improve their health, to educate them, to supply them with a decent standard of living through improvements in agriculture and housing, and to help them in establishing a democratic and equitable form of self government.

I was favorably impressed by the caliber of the men and women in this work. Of course there are a few inevitable exceptions and at the other end of the scale people who seem to stand out in their positions. I shall mention two at this point. I was told by my colleague, Dr. Marshall Wheeler, of the individual who flew with him and several others down to the Kapingamarangi Atoll to handle some legal matters. I shall call him La Feete (that was not his name). He carried a bottle with him and was in a drunken stupor on the plane. During the negotiations with the native chief and his aides he had his bottle out on the counsel table and would occasionally take a swig from it during the negotiations. On the trunk of a palm tree growing near the door of the galley where we ate on Ponape was this inscription carved by some discerning individual: "La Feete, Jerk, First Class."

In contrast I would like to mention Mr. Byron Bender, assistant administrator of education for the Marshall Islands District. Bender is a graduate of Goshen College in Indiana, and has done graduate work in Anthropology. When he heard that I was from Wooster the evening of our get-together at the Club on Majuro, he asked me if I knew Howard Yoder. I assured him that I did and that we both belonged to this august organization. It seems that Bender roomed with Howard's son in Goshen and has been a guest in his home here in Wooster. After the festivities at the Club, Wheeler and I went over to the Benders' home on Majuro for a midnight cup of coffee and some cake. With his young wife and two daughters, they have spent most of their married life, several years, in Majuro working in the educational program. Their home is half of a Quonset hut, well supplied with books, quite comfortable in so far as comfort can be obtained in that tropical, humid climate, their children delivered by native Marshallese doctors, their hearts and energy in the work they are doing. I liked young Bender, his enthusiasm for the work he was doing, his dedication to it, his wisdom for one so young. I happened to overhear a conversation the next day at table between Mr. May-

nard Neas, the district administrator, and some of the A. E. C. men who had flown down with us. He was speaking in highest terms of Bender's work among the natives. On his own initiative he has set up a hand-printing shop and is composing and printing textbooks for the schools. He is to be in charge of taking the Rongelap natives back to their home Atoll and reestablishing them there this fall. The Rongelap natives were evacuated from their home atoll in the summer of 1954, after they had received radiation from the fall-out in the big blast that summer. They have in the interim been on Majuro Atoll, and rather unhappy there. To me, one atoll looks as God-forsaken as another, but to native Marshallese whose ancestors have lived for many generations on that 3.07 square miles of palm and pandanus bedecked coral strand I suppose the adage "Be it ever so humble, there's no place like home" has a meaning. At any rate Bender has the job, among many others, of taking those people back home to the very adequate housing, western style at their request, which will take the place of the beautiful pandanus huts which they formerly occupied; yes, of course the U. S. foots the bill, but could they do less for a group of natives burned out in more senses than one. Needless to say the tail end of the evening which Wheeler and I spent in the Bender home was a fitting climax to what had gone before.

To be successful in the work they are doing, and in my opinion most of those whom we met were eminently successful, these people must have a sense of mission. They must be and they are dedicated to the job they are doing. They must be able to take it. By that I mean to live a life quite different from that which we live in the states; work in a climate with no change of seasons (the only difference on Ponape is that there is a wet season and a wetter season with an annual rainfall of around two hundred inches), a debilitating climate where the day temperatures are in the eighties and the humidity close to 100%. An outsider wonders what is to be gained by foisting western civilization onto a people whose culture seemed well adjusted to the environment. The U. N. workers see those problems even more clearly. This I know from very rewarding conversations with several of the personnel on Majuro and Ponape. There is also the constant frustration of wondering how long this project will be continued. They know full well, and they're not kidding themselves, that the U. S. is over there for one major purpose. These islands constitute what Hobbs has called Fortress Islands of the Pacific. We're in there because the islands give us potential military and naval bases, and who knows, with the evolution of up-to-date means of war how long such islands will remain of importance. The Trust Territory people must act and build as though this is a long term project, but none realize better than they that the project may fold up tomorrow. And the budget, compared to the billions of dollars spent at the Pacific Proving Grounds for military purposes is certainly puny. There are so many things that could be done on Ponape if they only had the money and personnel to do them. But I heard no complaints from the Trust Territory people on this score. I only heard this point very strongly put by Tom Hardison of the A. E. C. Many others in the A. E. C. feel the same way. It is heartening to see how some of these men go out of their way to see that the district administrators and others get the scraps from the military table, scraps which would rot on coral beaches otherwise, and it is heartening to see what these administrators can do with such scraps. I can only

hope that this Trust Territory project may be continued for many years, for it is my opinion that the goodwill invoked from the wise administration of such a microcosm experiment may well furnish a defending bulwark for democracy all out of proportion to the money invested. This, of course, is one man's opinion, and the opinion of one who feels strongly that the cold war will eventually be won by a constructive attack on the problems of providing a decent chance to live the good life for every human being on the planet. Incidentally this will involve an honest consideration of the greatest problem which faces us today, the problem of the limitation of world population.

Of course the peoples of the Marshalls and Carolines have been subject to foreign influence and domination for a period long antedating the second world war, and it is against this background that the Trust Territory personnel must work today, knowing full well that what they do will be compared with what went before. Between the first and second world wars the Japanese held a mandate over the Marianas, Marshalls and Carolines. To this island empire, already overpopulated and feeling the need for "*lebensraum*," the mandated territory offered a new potential for immigration, and the establishment of industries and agriculture to supplement the precarious economy of the home islands. During this period there were over 3000 foreigners, mostly Japanese, living in Colonia, the principal town in the north of Ponape. On the other side of the island in Matalanim (Madolenihm) province there was a settlement of over 2000 Japanese and Okinawans. There were more Japanese living on the island than the native population of some 8000. The Japanese developed commercial farming in the interior valleys, introducing the culture of rice and sugar cane. They established industries. To quote from Raymond E. Murphy in an article on "*High and Low Islands in the Eastern Carolines*":

In the years before World War II certain non-agricultural activities had developed in Ponape and Kusaie (the other high island of this district), but particularly in Ponape, in which the natives had little part. Commercial fishing was in the hands of Japanese using Okinawan labor. There was some small-scale manufacturing in Ponape, including fish processing, paper making, the making of starch from cassava and of alcohol from sugar cane, lumbering, soap making, ice making, cotton weaving and cigarette making. On both the high islands electric power plants were in operation. But none of these activities was in native hands.

However, the natives or at least some natives did enjoy some fringe benefits from the agricultural and industrial activities of the colonizers. Actually there was room and resources for both colonizers and natives. They were not stupid colonizers. They established health service for the natives, some schools and introduced many products of commerce for sale or barter, goods which changed the economy and way of life of the natives, whether it bettered them or not.

The earlier history of foreign influence in the islands can only be outlined briefly. While both the Marshalls and Carolines were visited sporadically much earlier by sea-faring explorers (they were first discovered by the Spanish ex-

plorer, Alvaro de Saavedra, in 1528–29) they were regularly visited by New England whalers during the period 1820–1850. No one seems to have a good word for the influence of the whalers. Hobbs writes of the Marshalls: “The whaling crews introduced the diseases of syphilis and tuberculosis and the vice of drunkenness with the usual results.” Henry Hedges, district administrator for Ponape, writes of that island: “contact with western whalers left disastrous effects on the lives of the people in this district, as in other areas of the Pacific during the middle 1800’s.”

About the middle of the nineteenth century Protestant missions, (the Boston missionaries) were established in both the Marshalls and Carolines. The islands were held by the Spanish during much of the latter part of the 19th century and their priests established Catholic Missions on both the Carolines and Marshalls. The Protestant missionaries reduced the local languages to writing, translated the Bible and taught many of the local populations to read and write. Today both Catholic and Protestant missions are flourishing on Ponape and in the Marshalls, and the natives are roughly evenly divided between the two churches. It is my impression that the civil administration maintains friendly cooperation with both religious groups. Thus on Ponape the school administrators have worked out a plan with the Protestant mission by which efforts will be supplemented and not duplicated in the education of the natives. We met the Catholic priest on Majuro and he seemed to be a person of tolerance and charm.

In 1899, following the Spanish American war, the Marshalls, Carolines, Gilberts and Marianas, with the exception of Guam in the latter group, were ceded to Germany for the small sum of 840,000 pounds (Hobbs). Germany lost the islands to the Japanese following World War I. Thus through the 19th and first half of the 20th century the natives of these islands have felt the impact of outside influence: American whalers, American Protestant missionaries, Spanish Catholic missionaries, Spanish, German, Japanese, and now American administrators. Whether one likes it or not the fact remains that they are engulfed in the tide of western culture and civilization, nor can one except the strong Japanese influence, which was essentially western in many respects. The Americans at Majuro and Ponape are committed to the task of superimposing upon and blending and coordinating some of the values of western culture with a native culture and economy seemingly in most areas of living well adapted to the environment. It is a challenging and at times frustrating problem. The program, both on the low islands of the Marshalls and on the low and high islands of the Carolines is designed to help the natives help themselves and as fast as possible native leadership is being trained to take over positions of responsibility. In the Marshalls at Majuro a hospital of several large quonset huts is at present staffed entirely by native Marshallese doctors, aides and nurses. The Marshallese have a hospital ship, the crew and staff of which all are Marshallese and which make the rounds of inhabited atolls, visiting each at least once every three months. Serious emergency cases may be flown to the Naval Base on Kwajalein or to Honolulu. Miss Elizabeth Hirst, hospital administrative assistant on Ponape, showed several of us through the hospital there. She had formerly been in a well-equipped hospital financed by the Rockefeller Foundation in China, and kept apologizing for the inadequate facilities and poor equipment in the local hospital. However, to the

layman it appeared that the Ponape Hospital was doing excellent work with modest equipment. We visited the T. B. ward, the leprosarium, and the general wards. Yaws, one of the worst scourges in these islands in the past, has been eliminated by the use of modern antibiotics. T. B. is now the worst disease among the natives, probably due to inadequate diet and the extremely humid climate. Serious cases in both Marshalls and Carolines are hospitalized. There were thirteen cases of leprosy in the Ponape hospital, none in the advanced stages; this disease seems on the way out with new drugs developed in recent years. Venereal disease is at present relatively rare in both island groups, though formerly much more prevalent. One cannot but feel that the introduction of western medicine has been a godsend to these people, whatever may be said of other phases of western civilization.

At Majuro, Tony Cruz, a native of Guam of Chinese extraction, with graduate training at Oklahoma A. and M. is in charge of the Agricultural Experiment Station. This is an Ag. station in miniature. We saw a good deal of Tony Cruz and were impressed both by his energy and with the problems which he faces. Coconut palms grow luxuriantly on all the low islands. In some of them taro swamps are maintained quite successfully. Papaya and breadfruit grow quite well. Tony has the task of trying to find other food plants which can be grown successfully in the salt-spray laden air and the sandy soil of the atoll islands. While Robert Louis Stevenson makes his point in the quotation read above on the paucity of animal and plant life on the atolls by a gross understatement, nevertheless there are few plants which grow here in any abundance. William Randolph Taylor in *"Plants of Bikini and other Northern Marshall Islands"* lists 38 dicots and 5 monocots in addition to grasses. To those of you who know something of taxonomic botany this is a restricted flora indeed. Atolls of the Southern Marshalls and Carolines would add very few new species. In contrast Sydney Glassman in his *"The Flora of Ponape"* lists several hundred species of the higher plants. I can say that from some experience in tramping over the motus of several Marshall Island atolls about a dozen trees and shrubs make up the bulk of the dominant flora. I will not burden you with a list of these plants. In contrast on several long hikes on Ponape I was impressed by the variety and luxuriance of the flora. Some of the Americans on Majuro try to grow a vegetable garden but when it comes to the culture of tomatoes, lettuce, etc., the results are rather discouraging. Most food plants can't take the salt air. Tony Cruz is breeding chickens, ducks and pigs in some numbers. Improved breeds of these will be released to the natives who have for a long time raised pigs and chickens, but with no attention to improving the stock. In the past the economy of the atoll islands has been based largely on cocoanut, shell-fish and fish. Taro is also an important food crop and is really the only crop which they culture. Cocoanuts grow wild and with little cultivation. Natives simply harvest the crop which is maturing the year round. I hope Tony Cruz is able to find some new crop plants with high yield on small land surface, but the task is a formidable one. Papaya, bananas, bread-fruit, and pandanus can be grown on the low islands, but all flourish better on the high islands.

On Ponape according to Murphy "between 40 and 45 species of wild and cultivated plants are used as food by the natives. The list for the atolls in this region

would probably not total more than fifteen." Furthermore the high islands are undoubtedly adapted to growing many plants not now found there. Thus an experiment station on Ponape has a real chance of developing a diversified plant and animal breeding program. We spent parts of several days at this station and were shown around by the head agriculturist, Edward Iwaniec. I can only mention briefly some of the things we saw: taro swamps, where many varieties from the several atolls and Ponape were being grown and crosses made to develop improved types adapted to certain local conditions, cocoanut palm groves where records were kept of yield and quality, planting of tea, coffee, cacao, and rubber as possible commercial crops for the island, pigs imported from Japan and being fattened on Ponape bananas, of which there are many species grown, a small herd of the new Brahma cross cattle from the King Ranch in Texas, and of course many exotic plants grown for ornament. One had the impression that Iwaniec and his corps of workers had a wide-open field for investigation in contrast to the limited future of agriculture in the Marshalls. However, at the present time, the atoll islands both in the Marshalls and Carolines produce much more per acre and support a much larger population per square mile than the high islands. Ponape is still mostly a jungle wilderness. This point can be documented best by figures: Ponape has a population of 120 per square mile of arable land. In contrast in the Eastern Carolines Mokil Atoll has a population of 878 per square mile of arable land, Pingelap of 1025 per square mile, Kapingamarangi of 817. The atoll islanders have reached and perhaps in cases exceeded the population which can be supported there. They are free, however, to migrate to the high islands, where life would be easier and more abundant for less effort. Some migration has occurred. It is not unusual for individuals, families and even larger units to migrate from low to high islands; practically no migration in the opposite direction has occurred. However, in spite of the more rigorous and precarious existence on the low islands the natives there for the most part are loathe to leave their homes for the easier life on Ponape and Kusaie. They claim to like the atoll climate better, though from my experience I can see little difference. In both places it is hot and humid.

It is my impression from observation, conversation and reading that the low islanders of the Marshalls and Carolines are somewhat more industrious than the high islanders of Ponape and Kusaie. But one must be careful about such generalizations. If this be true it is probably because with more limited potential resources on the low islands the natives have had to work harder and to chance more to survive. Thus I understand that atoll dwellers in general are better sailors. They do much of their fishing in the open sea and in the past traveled hundreds of miles in out-rigger boats from atoll to atoll, navigating by stick chart. They were formerly expert in knowledge of ocean currents as M. W. de Laubenfels has pointed out in an article on "*Ocean Currents in the Marshall Islands*" in the following words:

Every time a native sailboat was becalmed, its crew paid careful attention to the rate and direction of drift. If they succeeded in reaching land, they reported their experiences with great exactness. The older navigators thus accumulated a dependable store of information. Many

scientists who value only complex instruments may belittle the words of natives, but practical naval experts (for example Captain John Vest, former governor of the Marshall Islands) recommend serious reliance on them. Special mention may be made of Lokrap of Ebon Atoll, whom the present author regards as the greatest of the surviving wise old navigators. The younger Marshallese do not carry on the old skills but prefer rather to get a Diesel engine and forget about currents.

It seems unfortunate that the Marshall Islander of today has largely given up fishing, a major factor in their earlier economy. Now they work for the Trust Territory projects, or for the navy at about 25¢ an hour and buy canned fish at the grocery. It is one of the projects of the administration to encourage a return to fishing, but now with larger boats carrying reefers for refrigeration. The Ponapeans do some fishing but inside the reefs; in contrast the men of the Kapingamarangi village on Ponape (immigrants from the home atoll five hundred miles to the south) go fishing regularly both inside and outside the reefs with their fleet of outriggers.

On the Marshalls by far the largest industry is the production of copra, the partially dried meat of the coconut, which is used in making coconut oil, soap and other products. They have a native company, Mieco, Marshall Island Export Company, supervised at their request by an American, but run by Marshallese for the Marshallese. Last year they exported 12,000 tons of copra. At market price of \$175 per ton this runs into over \$2,000,000 gross, no small business for 11,000 natives living on less than 70 square miles of land. In the summer of 1955 we attended an outdoor movie on Majuro, a walk-in not a drive-in. We sat on the grass with the natives and used our rain-coats before the show was out. We saw a picture "Storm Over Tibet" featuring avalanches in the Himalayas and wondered how much of the picture was getting across to island natives who had no experience with snow or mountains. But they were attentive. On our visit to Majuro this past summer we were shown through the fine new Majuro Club building, put up by Mieco, designed and constructed by Marshallese from lumber they had imported from Japan. We left on a Thursday and the formal opening with appropriate celebrations was scheduled for the following Saturday. On the first floor was a movie theater accommodating between 1500 and 2000 with wide screen and modern projecting equipment, a restaurant and soda-bar and store. On the second floor were recreation rooms and a large Council Room where the Marshall Island Congress, with its upper and lower house, would meet. Certainly, the high island natives of Ponape have no such ambitious native company. A convenient bread-fruit tree near his dwelling furnishes the bulk of the family food the year round. While the crop is seasonal, the bread-fruit is preserved in various forms and used the year round as food. William Finale (native of Cleveland and the Educational Administrator on Ponape) told me that he lived for two months with a native family and that the diet was almost wholly of bread-fruit in different forms. He had meat only once in the two months. This of course, is a subsistence diet and not a properly balanced diet. But many Ponapeans live that way.

While the Ponapeans may be more shiftless than the low islanders because of

their more easily secured food supply, I don't want to imply that they are not capable of hard work, at least some of them. In the eight days we were on Ponape we saw natives bring coral on barges to the landing; grind it up; haul it by truck, spread and roll it down and surface a road approximately a mile long from the town of Colonia out to the Agricultural Experiment Station. It might be remarked that this road had a strong, fishy smell. Undoubtedly this would disappear as the coral animals decayed and the rain leached the organic material out. The Ponapeans were also putting up a new movie house but on a much more modest scale than that on Majuro.

Let us now consider very briefly the school system set up by the Trust Territory Administration. In the Ponape District there is an Educational Administrator, William Finale, and three American Assistants. In the neighborhood of thirty elementary schools are maintained, at least one in each of the five provinces on Ponape Island, several on Kusaie and at least one on each of the inhabited atolls. Under these administrators native teachers, invariably men, generally in their late twenties or thirties, conduct these elementary schools. Here children are taught to read and write in Ponapean, and in English. Arithmetic, geography and history are also taught. The better students from these schools are brought to the secondary school in Colonia on Ponape and as boarding students continue their studies. They may spend two or three years here with infrequent visits back to their homes particularly if they live on distant atolls. This school would correspond to the upper grades in our own school system. Some of the better students from this school then go to the Island of Truk to the PICS school (Pacific Island Central) where they get a high school education. From there a very few of the best students go to the University in Honolulu or to the British University in Suva in the Fiji's. At Suva the British maintain a first class Medical College and it is here that advanced laboratory technicians and doctors get their training. Plans are underway to move the PICS School to Ponape where it is to be located on a tract near the Experiment Station. Ground is probably already broken by now for some of the buildings. As I was walking in the Experiment Station grounds one hot, humid afternoon (the only kind they have the year round) I couldn't help overhearing an argument between Iwaniec, Ag. Station Head, and the chief surveyor for the road to be cut through the station grounds to reach the school. The argument was over where the road should go. Tempers were a little short. One school of thought had it that the road should be as direct as possible, the other that certain exotic trees and shrubs, not easily transplanted or re-grown should be saved regardless of where the road had to go. I was for the second position but didn't wait to hear the outcome as it was none of my business.

After thirty pages of introduction we haven't yet gotten around to what was to have been the main thesis of this paper: the natives of the Marshalls and of Ponape, who they are, where they came from, something of their customs and manners. According to Thrum's Hawaiian Annual and Standard Guide, the Recognized Book of Authentic Information on Hawaii, "The Polynesians were the descendants of a family or tribe of Caucasian people who left their original homeland somewhere in India and wandered through Malaya to the island groups of the Pacific. In their travels through Asia, they intermarried with Ma-

layans and Orientals. From the inhabitants of the southeastern islands of the Pacific (Melanesia) [*sic*] they also acquired darker blood, so that by the time they reached the Society Islands, now considered the ancient homeland of the Polynesian, they were a light brown people." If the thesis of Thor Heyerdahl in *Kon Tiki* is correct they also received an infiltration of American Indian blood from Peru, but of course the American Indians were originally migrants from Asia, so this does not notably change the origin of their racial stock. The Melanesians are thought to have a strong admixture of primitive Australoid blood, in addition to Asiatic strains, and the Melanesian aborigines have kinky hair and darker skins, which favor this thesis. The Micronesians would seem to have a mixed ancestry of both Polynesian and Melanesian origin. And one does not have to be an anthropologist to see in individuals of both Ponape and Majuro atoll evidence of blood transfusions from their earlier Western and Japanese overlords. I gathered from conversations with personnel both on Majuro and Ponape that extracurricular activities between natives and administration personnel are at present neither condoned in the social rules of order nor even engaged in clandestinely. Of course, during the occupation of the islands in World War II things were different.

The people of Majuro Atoll in the Marshalls and of Ponape in the Carolines are on the average people of medium height, stout in body build, with straight, coal black hair, brown eyes, and broad flat noses. Superficially at least they are friendly, greeting you with smiles and a friendly salutation either in town or on a trail far from town. When I mentioned this to the anthropologist on Ponape he suggested that this might be an act put on by natives to keep on the good side of the Americans. My answer to this was that I always spoke to all children I met. The American children on Ponape were likely to be reserved; they might return the salutation but they didn't often smile. In contrast the Ponapean children almost always smiled broadly. I don't know that I won the argument. Possibly my bizarre appearance was sufficient to elicit the incredulous smile of a native youngster. But I still hold to the hypothesis that among these people there is a larger proportion of pleasant extroverts. Mr. Neas, administrator on Majuro, told us that there were no crimes of violence, murder, rape or major thievery among the Marshallese.

Perhaps rape is less common in the islands because of the somewhat more casual relationship of the sexes. It is significant to note that in both the Marshalls and the Ponape District there is a matrilineal system of land inheritance. This was explained to us as being a system which fitted in well with social customs where it is always easy to determine the mother of a child but not so easy to know who the father was. In the Carolines I am told that each young man of marriageable age has a love-stick with unique carvings on it. If he fancies a certain maiden he goes around to the hut where she sleeps and pokes the love stick in. Apparently she can recognize the carvings on the stick in the dark and if she favors the suit she pulls on the love-stick and the suitor comes in. If he doesn't get the green light I suppose he goes off to the hut of No. 2 on his list. I don't know how much of this is fact and how much fancy. But I do know that Chaplain Hoppe, from Eniwetok, a member of our party, had a native boy as a guide on one hike which he took. He questioned the boy on this matter and he replied that

he had the love-stick and the girl picked out but so far hadn't gotten up the nerve to try the experiment. On the eve of our departure from Ponape the very attractive waitress at the galley presented a love-stick (unfortunately not to me but to the one unmarried member of our party). She assured him that this was just a souvenir and had no further significance. It would seem that the love-stick custom has some phallic significance but I am no anthropologist.

If a married man goes off to another atoll to work for a few months it is not unusual for him to take another consort on that atoll and for his wife to make an appropriate connection back home. I noted in Glassman's *"The Flora of Ponape"* that between 30 and 40 plants were listed as drug plants used by native doctors, and among these a surprising number were used as contraceptives. I don't know how successful these are but I do know that the anthropologist on Ponape, Mr. Richard Emerick, told me that native families there were likely to be three or four children. To him the low birth rate was a puzzle and a problem for future investigation. Native populations are now on the increase after a long period of depression, but this is due to introduction of western medicine and not to high birth rates.

What kind of clothes do the natives wear? I was asked this question repeatedly after my return from the Marshalls. I could only say then that the women of Majuro were the most dressed women I saw anywhere on my travels, with more bare epidermis on display on any beach in America or for that matter on any downtown street or in any grocery store in mid-summer than one would see in the Marshalls. Both men and women wore cheap cotton clothes, the women with "mother hubbards" which certainly concealed the form quite well. We were told that this was a result of the Puritan, prudish influence of the Boston missionaries. On Majuro male children often went naked; I saw a group of these boys diving off the dock and it reminded me of the old swimming hole back in Southwestern Ohio where I grew up. But females of the same age were always clothed.

What kind of homes do the natives live in? Both on Majuro and Ponape most of the natives live in shacks constructed of odds and ends of lumber and sheets of galvanized iron. They are ugly, not a redeeming feature except that I suppose they keep the rain off and there's lots of rain. But I could not help but contrast them with the beautifully and elaborately constructed native houses on Rongelap atoll, and the less elaborate thatched houses in the Kapingamarangi village on Ponape. The Rongelap houses, abandoned when the natives left in the summer of 1954, are being replaced by western style homes. Since they were abandoned we had the chance to inspect them closely. The thatching is of pandanus leaves, with mid-ribs from palm leaflets used as binding and supplemented with cord of palm fiber. The work is elaborate, beautiful and durable. From the inside the pattern is perfection. These houses with their slanting roofs, broad, overhanging eaves, and with side walls rising only about four feet and open above are admirably adapted to the climate. In the Kapingamarangi village on Ponape the roof thatching is of palm leaves (pandanus is not so abundant here) and much less elaborate but quite adequate. A few of the houses in this village and the church are constructed of lumber and galvanized iron. I remarked to the native teacher in the village, a young man who spoke some English, that I liked much better the looks of the native huts. He said in reply: "But we like the other build-

ings better. It's easier to lay on a sheet of corrugated iron than to make the thatching." He told me, however, that the thatch would last about five years and one of the construction men on Ponape told me that the corrugated iron would last about the same length of time. Native boats, native clothes, native houses, soon to be a thing of the past. But quite possibly when and if we pull out of this territory the natives will have to go back to their old ways. Perhaps, who knows, the natives of Yap and Kapingamarangis are the wise ones. They have best retained the old cultures and would have less adjustment to make in case the ships and planes no longer came to their shores.

In the Ponape Co-op run by Joe Riddle, who lives with his family close by in the old Japanese Geisha house, natives can and do buy all kinds of western goods. Utensils of rubber, buckets and dishware are very popular. They have a much longer life than those of metal which soon rust out. It's easier to buy cord at the store than make it out of palm fibers. Nails are so much better than palm fiber and wooden pegs when it comes to constructing houses. The standard wage for labor on Ponape is 15¢ to 28¢ per hour. No doubt if I were a native Ponapean on that wage I would be buying utensils and cotton goods in the co-op rather than making cloth out of native grasses, and utensils out of cocoa-nut shells and sea-shells. More than once I saw what seemed a symbolic picture, a native woman far back in the bush, industriously running a Singer sewing machine, no doubt constructing articles of clothing to cover her family. But why? She'd be more comfortable with fewer clothes. I sometimes wonder if the human rat-race which we call western civilization is the final answer to the age old problem of the good life on the planet earth. Just think of buying a Singer sewing machine on a 25¢ per hour wage. The Cadillacs aren't there yet but surely they're on their way.

I want to close this rambling discourse with a few word pictures as substitutes for the kodachromes I didn't take because we were not allowed to carry photographic equipment into the Proving Grounds and therefore to other places we visited.

A native luau (feast) on Ponape. Bill Finale and his charming wife, Betty, had invited us to dinner at their home on Ponape, Monday evening, August 27th. In the meantime Bill had discovered that his native teachers on Ponape were arranging for a luau in honor of three young men of Ponape, returning from the University of Hawaii in Honolulu. So the two festivities were combined. About dusk Henry Takeshita, a Japanese American originally from Honolulu and administrative assistant (if you wanted to know anything ask Henry) called at the crew's quarters and picked us up in that ancient truck we had ridden on many times before. A few minutes later we were in the living room of the Finale home, when drinks were being served, and introductions made to native teachers and the guests of honor, the Honolulu boys back home. Soon we were out on the lawn, brilliantly lighted with arc lamps. On the lawn were spread big pandanus mats; in the shadows could be seen banana trees, palm trees and other luxuriant tropical vegetation; a buffet table had been prepared; a table covered with huge banana leaves as table cloth; on the table was an unbelievable array of native dishes. I am no gourmet, only an amateur in the arts of the delicatessen and the cuisine, but I can think of only one comparable spread in my experience, a wedding feast in Egypt in the home of a wealthy land-owner long before the days

of Nasser. On that table lay a half-grown pig, roasted and bisected down the middle, a huge plate beside it containing the roasted extremities of pigs' legs, jaws and parts of heads; some of the jaws looked too much like dog to suit an amateur anatomist, so I settled for a leg; breadfruit in three different guises, yams, baked bananas, taro root, cocoanut in different forms including a pudding; rice in monumental heaps and several other items. We helped ourselves, buffet style, and sat on the pandanus mats. Before we ate, natives made the rounds and placed on the brow of every banqueter a wreath made of exotic tropic flowers. During the meal cocoanuts were passed and from them we drank the milk. Coffee and drinks were also served. As table companion I was fortunate in having Connie Hedges, wife of the administrator, and she regaled me with tales of their years on Bora Bora near Tahiti, and of the experiences in taking natives of Tahiti and Typee back to Chicago. Ponape skies were kind and it didn't rain that night. To me it was an experience out of the Arabian Nights. I could only wish that my wife had been there for I'm sure her capacity for the enjoyment of the romantic and the exotic is greater than mine. But such is life. What do I think of native food? It's flat, tasteless, and not sufficiently seasoned. I'd settle any time for the kind of food served us daily at the A. E. C. base on Parry Island.

Saturday night, Party at Colonia Club on Ponape. What's better than an evening of good conversation, particularly when the setting is a Clubhouse on a far off Pacific Isle, and the participants young intellectuals eager to match wits with outside visitors of which there are too few in that faraway place? I'll tell you what's better: a beautiful young Japanese American from Honolulu, dancing an authentic hula at 2:00 A.M. that "evening" at the club. It's the arms, the hands, and the fingers that tell the story and not the hips, believe it or not. Well, that's something to park away in the cortical folds along with the first view of Ponape from the air.

Long Hike into the Hinterland on Ponape. On the morning of August 28th Marshall Wheeler and I took a long hike, about ten miles, from the province of Net into the province of Uh on Ponape. Afterwards Wheeler told the others that there was only one thing wrong with taking a hike with Spencer: he walked too fast. But that wasn't fair, for Wheeler had coral cuts on both feet, which I didn't know of at the time but which must have been painful. Then I had gotten into condition by tennis earlier in the summer. I had earlier made a trial run on this trail. Along the trail we passed two mountain streams where Ponapean women were busy beating the dirt out of the family washing with clubs. I suppose this treatment gets the clothes clean, but it must be hard on buttons, and fabric. I've seen the same technique used in Egypt and I suppose it is universal custom in the tropic belt around the world. A little later we crossed a meadow on the border of which we saw several species of tropic birds (a few species are found in Ponape and nowhere else). One is a brush-tongued honey-sucker, a specimen of which the Riddles have as a pet. They call him Einstein, and when I asked Riddle why he didn't take Einstein to the parties he said that he would be bored at the level of the conversation. A little further along the trail we saw huge clumps of bamboo, and further along on higher ground between two mountain ranges huge tree ferns, some forty feet in height; still further a species of palm, the individual leaves of which were between thirty and forty feet long and the petioles of the

leaves at the base almost big enough to fashion into a dugout canoe; unfortunately, not being a botanist I could not check the species from Glassman's checklist of the plants of Ponape. The trail led at one place across a very substantial bridge, built by the Japanese over a lagoon; most of the trail found black volcanic rock underfoot, sometimes slippery, always rather rough and not too easy hiking. But the changing scenery as we hiked back along a valley trail between the mountain ranges, with rank tropical vegetation on either side, native huts for the most part solitary and not in villages, natives on the trail and at work near their homesites, all this was an experience worth more than the primary object of the journey. We decided later that this was the main-line trail from the north to the south of the island and a few hundred thousand dollars would turn it into a passable highway.

A visit to the Kapingamarangi Village on Ponape. This was the high-point of my stay on Ponape. Actually I made three visits to this village, but we will merge them into one. Let's make the trip together, but we'll take Chaplain Hoppe along, for he has been out there several times doing some oil painting and he knows the ropes. As we leave the crew's quarters with the tropic sun shining brightly on a late August morning some will carry rain-coats and others will not. I belong to the latter school, for I have decided that it's easier to get wet from the rain and let my dacron pants dry out than to wear a rain-coat during a shower and get wet from sweat. We'll take the road up past the administration building and the ball diamond, past the hospital, the garage and the filling station, all important landmarks in this community. Before we come to the secondary school and the experiment station we take the road off to the right, past a very fine planting of pineapple and up onto the high ground overlooking the lagoon, beyond which can be seen Jokaj (Sokehs) Rock. We simply follow the trail and after passing several Ponapean houses of the usual scrap lumber and galvanized iron construction we come upon a few thatch roof houses. A few minutes later we are walking down Main Street, Kapingamarangi Village. These people are not Ponapeans. They have moved here from the home atoll five hundred miles to the south. There are about 200 natives in the village and four hundred more on the atoll. That's all the Kapingamarangis there are. Yet they have their own distinct language, quite different from Ponapean, and they have their own culture also quite different. Hoppe knows proper protocol. He takes us first to the long-house where we meet the head-man of the village. The long-house sounds like a chapter out of Melville's *Typee*. This is the biggest thatched building in the village. The sides are open; there is a raised board floor. Here only men congregate, except on very special occasions. That day there are seventeen men, young and old, in the long-house. Most of them are busy making fish-nets. A few of them are lying around, evidently taking a rest. The nets vary in size from one which must be thirty or forty feet long with large mesh, two inches in diameter, to delicate one-man throw nets, with a mesh about one-half inch diameter. Finished nets are also hung around the long-house. The chief does not speak English, but fortunately the young Ponapean teacher, who runs the school in the village, is there and he can answer some of our questions about fish nets and other things. I attempt to duplicate the tatting technique of one of the net-makers, and my awkward movements mildly amuse the onlookers. Either mystify a native by magic or make him

feel superior by obvious awkwardness in a skill in which he is expert. Soon cocoanuts are brought in and one of the young men makes a few deft slashes with a machete and has them opened at one end. We take one and drink the cocoanut milk; there must be a quart of it, mildly sweet and cool, a good refreshment for a parched palate on a hot day. The chaplain then suggests that we visit the church, which we do, walking through the back yards of villagers on the way and keeping our eyes open for the sights, including (as you have guessed) Kapingamarangi women in their native costumes, often soon supplemented by a swath of cloth. The church is a wooden building, smaller than the long-house, open on the sides but with iron bars. There are wooden benches for the communicants; seating capacity is about 150. Having visited the church and the long-house, where village council meetings are held, and met the head-man of the village, we are free to wander about through back-yards and peek into native houses. At least this was the Hoppe theory and it certainly gave us a fine opportunity to view native life. In one house there was a young mother, swinging her infant tied in a hammock. At the backdoor of another house we saw a young woman grating cocoanut. To do this the native sits astride a stool shaped like a saddle. In place of the horn on the saddle there is a projection carrying either a jagged piece of shell or a piece of metal with jagged edge, purchased at the Co-op. A half cocoanut is rubbed over this rather deftly and fine grated cocoanut results. In one back-yard pandanus leaves were spread out to dry in preparation for weaving them into mats. Women were cooking over open fires. Water was drawn from a stone-lined well near the center of the village. Large fish were hanging up near some huts. Children were bathing by pouring water over themselves, dipped from empty gasoline drums, set under the eaves of thatched huts to catch rain-water for washing and bathing. A new thatched house was being constructed for a newly married couple. I actually helped to raise the framework of the roof onto the stout poles set in holes in the ground. My help was not needed as there were about fifteen natives there to do the job. The following day a revisit of the village found the young husband alone starting on the laborious process of constructing the thatch. The wife was a pretty girl of about twenty years. Incidentally, the Kapingamarangis are Polynesians and somewhat lighter in complexion than their Ponapean neighbors. The day previous to this visit I had seen several natives sawing up the carcass of a beef in the village. I like to think this was in preparation for the wedding feast, and probably it was. We climbed down the steep path along the cliffs to the edge of the lagoon where we found the Kapinga boathouses. Perhaps a dozen big out-rigger canoes were housed there under thatched roofs, in preparation for trips both inside and outside the reefs to use the nets we had seen in construction. We were told that the big nets lasted only a few weeks, but the smaller throw nets might last for a couple of years. The nets were being constructed of cord, bought at the Co-op. That's easier than making the cord out of palm fiber. You'd probably do the same if there were a Co-op nearby. We were impressed with the neatness of Kapingamarangi yards; carefully kept sand and gravel paths, patches of grass with no weeds in them, quite in contrast to the rather slovenly appearance of the surroundings of a typical Ponapean home. But I am loathe to draw the obvious conclusion that low islanders are more thrifty and neat than high islanders, for certainly the yards

of natives on Majuro were as ill-kept as those of the Ponapeans. Yet they were low islanders. When the villagers heard that Wheeler and I were flying down to their home atoll, five hundred miles to the south, on the following morning they were quite excited. Through the school-teacher as interpreter they asked if they might send a packet of letters to their relatives there. We, of course, said we would be glad to take the letters. The following morning one of their number was on the dock, from which the picket boat left, with a big stack of letters. Unfortunately I was bumped for a V.I.P. on this trip, so never got to see the atoll. But Wheeler delivered the letters and brought a big pack back, as well as models of outriggers and some fine basketware given him in appreciation by the chief of the Kapingamarangis.

An Evening at the Club on Majuro. It is an evening in late August, 1955; our party is lounging in easy chairs in the Majuro Club. We are listening to a dozen or so of the Trust Territory boys discuss their several problems, including absent and incoming personnel, friendly gossip if you wish. The native bar-boys pad softly about, taking orders and mixing drinks. The surf from the open sea beats on the coral reefs just outside the screened but open siding at our backs. The temperature is down to the comfortable seventies with soft sea breezes wafting gently. Except for the lack of British accent, just like a chapter out of Kipling. Same setting, late August 1956, but the restful, out-of-this world atmosphere completely spoiled by the addition of one new environmental factor. Guess what it is? An American juke-box blaring jazz tunes all evening on a constant diet of nickels which you may be sure I did not supply. Let us consider it a symbol of western influence on a far-off tropical isle.

I close with a quotation from John Wesley Coulter in an article entitled: "*The United States Trust Territory of the Pacific Islands*":

The aspiration of native peoples for self rule is one of the factors of disquietude in the world today. It is not likely that any of them could maintain independence. They have to be educated in their own best interests and trained to look after themselves as far as possible. The situation demands from the advisors to native authorities training, knowledge of the psychology and history of the people and constant skill and attention to determine when to interfere and when to leave well enough alone, when to hasten the development of more advanced methods of government and when to advise a slowing down of processes too rapid to be assimilated. There are no pecuniary rewards to be hoped for by those nations charged with trusteeships. A trustee is, in terms used thousands of years ago, his "brother's keeper."



Darwin's Influence on the Study of Genetics and the Origin of Life¹

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It is customary for geneticists to point with pride to the fact that twentieth century genetics has supplied the crucial missing elements in Darwin's theory of evolution by natural selection. But what about the reverse problem? How has modern genetics been influenced by Darwin? It is this question that I propose to discuss.

I suppose that among all the diversity of subjects on which Darwin wrote, his writings on genetics appear to be the most confused to the modern reader. Consider his works on coral reefs, on earth worms, on heterostyly in plants, on self sterility mechanisms in general, on barnacles, on the habits of climbing plants, on orchids; these are remarkably perceptive and their essential correctness has been confirmed by modern work.

On the other hand, his vacillation about Lamarckism, his hypothesis of pangenesis, and his astonishing credulity in accepting folk lore and anecdotes makes his writings on heredity seem among the weakest and most puzzling of his work. Yet modern genetics owes a tremendous amount to Darwin.

The reason, of course, is the notion of change by natural selection. Paradoxically Darwin's great influence on modern genetics has come, not from his writings on heredity and variation, but from his idea that natural selection, by multiplying variations, is sufficient to account for the diversity of living forms.

INBREEDING AND HYBRIDIZATION

With his characteristic patience and attention to detail, Darwin did a monumental study of the effects of inbreeding and what we now call heterosis. He published the results, also characteristically, as a large book.

After trials on many plant species, he noted that inevitably inbreeding led to a decline in height, weight, and vigor. He also realized that the effects of inbreeding were gradual and cumulative over several generations, but that there was immediate recovery on outcrossing. A similar increase in size was seen in variety crosses.

All these results are familiar to modern geneticists and easily understood as consequences of mendelian theory. Darwin also noted two other effects of inbreeding that seemed very puzzling to him—the prepotency of inbred lines in outcrosses (now understood to be a consequence of homozygosity and dominance) and the uniformity of plants within inbred lines. He called attention to the fact that the flower colors in *Petunia* and *Dianthus*, which were ordinarily heterogeneous, became remarkably constant with inbreeding; so much so, that

¹ Paper Number 725 from the Department of Genetics. This is a slightly modified version of a talk given at the Symposium on "Darwin's Impact on Biology" sponsored by the Society for the Study of Evolution.

his gardener had no difficulty in recognizing inbred strains by this fact alone. Darwin says (1): "As such cases of flowers becoming uniformly colored without any aid from selection seems to me curious, I will give full abstract of my observations."

Darwin's results and conclusions on crossing still stand up as empirical observations. In his own words the general conclusions can be summarized (1): "The most important conclusion at which I have arrived is that the mere act of crossing itself does no good. The good depends on the individuals which are crossed differing slightly in constitution, owing to their progenitors having been subjected during several generations to slightly different conditions, or to what we call in our ignorance spontaneous variation."

All Darwin lacked was an underlying explanation. The results of inbreeding and crossing follow so directly from mendelian theory that we find it hard now to put ourselves in Darwin's place of not having this knowledge. He did a remarkable job in seeing the generalities of this important phenomenon which, because of hybrid corn, is probably genetics' greatest economic contribution.

Realizing, as he did, that inbreeding leads to decline in vigor and is generally harmful, Darwin was fully prepared to find inbreeding avoidance systems and his studies of self sterility mechanisms followed naturally as a consequence of his knowledge of inbreeding and his theory of natural selection. He also noted the sterility that is a barrier to species and variety crosses. Again, I shall quote a pertinent passage (1): "It is an extraordinary fact that with many species, flowers fertilised with their own pollen are either absolutely or in some degree sterile; if fertilised with pollen from another flower on the same plant, they are sometimes, though rarely, a little more fertile; if fertilised with pollen from another individual or variety of the same species, they are fully fertile; but if with pollen from a distinct species, they are sterile in all possible degrees until utter sterility is reached. We thus have a long series with absolute sterility at the two ends;—at one end due to the sexual elements not having been sufficiently differentiated, and at the other end to their having been differentiated in too great a degree, or in some peculiar manner."

It is interesting to note that Darwin took the view, so ably argued in recent years by Muller, that hybrid sterility is incidental to evolutionary divergence, not ordinarily selected as an isolating mechanism *per se*.

PANGENESIS

In many ways Darwin's most unfortunate hypothesis was the notion of pangenesis, put forth in great detail in "The Variation of Animals and Plants Under Domestication." Yet, despite the general discrediting that it now receives the hypothesis is worth a closer look. It *did* account for a number of diverse facts. Throughout his life, Darwin was always looking for ways of bringing seemingly unrelated facts under a common viewpoint.

According to this notion "gemmules" were produced in various parts of the body and eventually reached the germ cells where they carried information from various somatic tissues to the next generation. This provided a formal explanation for Lamarckian transmission of effects of use and disuse.

Darwin apparently was never quite satisfied with this idea. He even had trouble with the name itself as the following quotation from a letter to Huxley attests (4): "What I called *pangenes* means that each cell throws off an atom of its contents or a gemmule, and that these aggregated form the true ovule or bud, etc. Now I want to know whether I could not invent a better word. *Cyttarogenesis*—i.e. cell-genesis—is more true and expressive, but long. *Atomogenesis* sounds rather better, I think, but an 'atom' is an object which cannot be divided; and the term might refer to the origin of atoms of inorganic matter. I believe I like pangenes best, though so indefinite; and though my wife says it sounds wicked, like pantheism; but I am so familiar now with this word that I cannot judge. I supplicate you to help me."

Furthermore, Darwin was always careful to label the pangenes hypothesis as provisional (2): "I am aware that my view is merely a provisional hypothesis or speculation; but until a better one be advanced, it will serve to bring together a multitude of facts which are at present left disconnected by any efficient cause."

One of the most definitive tests of the hypothesis was made by Galton who did several experiments that failed to show any inherited changes consequent to blood transfusions (5). Darwin did not regard this as a definite refutation however, pointing out that pangenes applies to plants as well as animals and that, although he certainly would have expected to find gemmules in the blood, this is not a necessary part of the hypothesis (2).

The hypothesis of pangenes deserves mention today for another reason. Currently we hear a great deal about self reproducing particles. Darwin thought of his gemmules as such particles. He was aware of the multiplication of the causative agents of smallpox and rindepest and suggested that gemmules might multiply the same way.

Another aspect of the pangenes hypothesis is that it provided an "explanation" for the still unsolved problem of communication between different organs of the body. How, for example, is the right kidney instructed to grow when the left has been destroyed? Hypotheses not too dissimilar to pangenes are currently invoked.

DARWIN'S VIEWS OF LAMARCK

It is of interest to see how Darwin's views of Lamarck changed during his life. In 1863 he wrote to Lyell (3) referring to Lamarck's book as "what I consider, after two deliberate readings, as a wretched book, and one from which (I well remember my surprise) I gained nothing." But with successive editions of the "Origin of Species" more and more Lamarckian ideas came in.

I think there are three principal reasons for this. First this was the prevailing notion at the time and Darwin was keenly aware of the thoughts of his contemporaries.

A second reason is what seems to be an amazing credulity on Darwin's part. He accumulated large numbers of isolated examples in his notes and these included folk lore and anecdotes about the inheritance of environmentally induced effects. His magnificent ability to consider a great diversity of facts and ideas simultaneously, and to bring them all under a common point of view here played him false—for too many of them were wrong. Darwin was caught in

what he himself realized was a serious problem, for he once wrote (6): "False facts are highly injurious to the progress of science, for they often endure long; but false views, if supported by some evidence, do little harm for every one takes a salutary pleasure in proving their falseness."

The third reason is the most interesting. Because he subscribed to the blending theory of inheritance current at the time, he greatly exaggerated the amount of new variability that would be required to maintain the population in a state sufficiently variable for selection to occur. Fisher (7) has shown that the variance is decreased by exactly half each generation with blending inheritance, whereas with mendelian inheritance a very low rate of mutation is enough to offset any effects of selection and random extinction.

Although Darwin does not appear to have been aware of the quantitative aspects of the problem, he must have had at least a qualitative notion. I believe he would have been highly receptive to Mendel's work for, as Fisher has noted, he once speculated in a letter about the possibility of particulate inheritance (4): "Approaching the subject from the side which attracts me most, viz., inheritance, I have lately been inclined to speculate, very crudely and indistinctly, that propagation by true fertilization will turn out to be a sort of mixture, and not true fusion, of two distinct individuals, or rather of innumerable individuals, as each parent has its parents and ancestors. I can understand on no other view the way in which crossed forms go back to so large an extent to ancestral forms. But all this, of course, is infinitely crude."

Today the discussion has shifted to new grounds. Within the framework of mendelian theory, what maintains the variance? Is it largely recurrent mutation to deleterious alleles with occasional polymorphic loci? Or is the typical locus heterotic as Wallace and Dobzhansky have suggested? How much potential variability is tied up in linked combinations? What is the role of the population structure in maintaining variability?

The question of what it is that maintains variability is still with us. The difference is that whereas Darwin had no explanation, we now have too many. Because variability is so easily conserved in a Mendelian system, there are several mechanisms any of which could be sufficient.

NATURAL SELECTION

The greatest contribution that Darwin made to genetics is, of course, the same as the great contribution that he made to biology in general—natural selection. The idea is at once so simple and self evident, so general and all pervasive, and so thoroughly taken for granted that there is very little for me to add. It has given a historical meaning, a perspective of depth to all biological properties. It provides a new reason for the study of mechanisms of inheritance and variation.

Usually the person most willing to accept and use a hypothesis is its inventor. Not so in this case. While Darwin was cautious in applying his notion, modern geneticists have paid Darwin the honor of being more doctrinaire in accepting his theory and extending it to new situations than he was. We are much more likely to regard selection as the sufficient explanation, the first factor to be ruled

out in any novel situation. For example, we no longer even stop to ask why domestic animals are more variable than their wild forbears—a subject to which Darwin devoted a two-volume treatise. Whereas he was unwilling to attribute this to simple selection, and looked for other mechanisms, we now regard it as entirely due to man's preservation of variants that would not have survived in nature.

We now emphasize that selection operates at many levels—between cells, between organisms, between demes, between species. But, although the ideas are now much more refined and quantified, thanks to Fisher, Haldane, Wright and more recently Malecot and Kimura, Darwin was aware of many of the complexities. For example, he considered the evolution of sterile castes in a bee colony as an example of inter-population selection. Strangely, he overlooked the important evidence this offers against Lamarckian inheritance.

Current selection theory assumes, much more than Darwin did, that much selection is wasted in that it has very little permanent effect on the genetic makeup of future generations. Darwin realized that not all variants were hereditary, but he had no way of knowing how frequently even genetically determined variants would not respond to selection.

DARWIN AND THE ORIGIN OF LIFE

Darwin's name is not ordinarily associated with the question of the origin of life, and I think he would want it that way. He argued strongly against some of his opponents who were maintaining that it was pointless to discuss the evolution of life without knowing its origin. He maintained, as almost all contemporary scientists would, that one doesn't have to know final causes to study immediate causes.

In fact, Darwin was quite inclined in his early years to ignore the subject of the origin of life. In 1863 he wrote to Hooker (3): "It is mere rubbish, thinking at present of the origin of life; one might as well think of the origin of matter."

Yet, Darwin has made a contribution to contemporary thinking about the origin of life in two quite different ways. The first of these is a remarkably perceptive statement made in one of his letters.

Until recently biologists have largely held to what Hardin (8) has called the autotroph theory of life's origin. It seems at first glance to be most reasonable to assume that the earliest forms of life made their own organic matter and had simple nutritional requirements. For, where do organic chemicals come from? From living organisms, of course. *Ergo*, the earliest forms had no organic compounds to live on. Furthermore, as George Wald has said, Wohler's synthesis of urea didn't prove the contrary—it only showed that a human being can make urea externally as well as internally.

But since Haldane's essay in 1925 the idea that the original forms were heterotrophs has gradually become accepted—thanks to Oparin, Horowitz and others who have added the needed details. But Hardin has noted that Darwin himself understood the essential point. Haldane's famous essay was anticipated in part by Darwin a half century earlier, when he wrote (3): "It is often said that all the conditions for the first production of a living organism are now

present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc. present, that a protein compound was formed ready to undergo still more complex changes, at the present day such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed."

Compare this with the following extract from Haldane's essay (9): "In this present world such (organic) substances, if left about, decay—that is to say, they are destroyed by microorganisms. But before the origin of life they must have accumulated until the primitive oceans reached the consistency of hot dilute soup. Today an organism must trust to luck, skill, or strength to obtain food. The first precursors of life found food available in considerable quantities for existence."

Both writers were ahead of their time, and their views were not until recently part of current biological thinking.

What is the essential feature of life? Muller as early as 1921 (10, 11) argues most convincingly that life depends on self reproduction, of course, but more uniquely on the ability to multiply variants. That is, life must have both self copying and mutation, with the mutant types being capable of copying *themselves*.

It is not necessary that the earliest forms be able to copy themselves accurately. In the beginning the probability of a correct copy need not have been much higher than that sufficient to offset purely thermal or thermodynamic degeneration. There may have been many more mistakes than correct copies. In this sense mutation may have been anterior to self reproduction in life's history. Natural selection would favor those whose ratio of correct to wrong copies was greatest—that is selection would lower the mutation rate.

Contemporary implication of DNA as the genetic material means that a relatively simple substance may have self copying properties.

I want to bring Darwin into this by suggesting that a meaningful, and perhaps useful, definition of life is that it is a system that permits the operation of natural selection.

I want to push natural selection as far back as possible into what is generally regarded as the pre-living, chemical realm. If it is generally agreed that macromutation is inferior to successive micromutation as an explanation of organic evolution, why not use the same thinking for pre-organic? An example of such thinking is Sagan's (12) discussion of systems that favor the accumulation of polymers in preference to monomers.

Now that space science is beginning we can start to answer questions about the nature of life on other planets. Soon the Arrhenius hypothesis of inter-planetary travel of spores can at last be tested. Is life (i.e. systems undergoing natural selection) on other planets chemically entirely different from ours? Or is DNA the basis of all self reproducing systems, i.e. is the primacy of DNA a chemical necessity or a historical-biological accident? I should like to record here my personal hope that life on other planets will turn out to be of independent origin—this will make the coming science of space biology a lot more interesting.

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Tumors in *Drosophila**

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Few students of genetics have failed to be intrigued by the hereditary, melanotic tumors in *Drosophila*, which have been known since the report of Bridges in 1916 (4). Stark (89-95) was responsible for the most extensive early work on them and drew an analogy between them and lymphosarcoma. Since that time a number of workers have isolated and examined strains bearing tumors (2, 6, 10, 28-32, 38, 45, 51, 53, 56, 65, 83, 85-87, 89-96, 101, 104) which occur spontaneously and sporadically in diverse populations and species (8, 69, 70). Matings of individuals with these tumors often show them to be hereditary. Our investigations have utilized only those which are inherited, since induction of the tumors by injections (25-27, 50) is beset by the difficulty that injury in insects elicits a response which may be indistinguishable from the tumors (88).

MORPHOLOGY OF THE TUMORS

The melanotic tumors (55, 60, 77, 78, 97) appear very early in larval life, and those we have studied contain two types of cells. The first are large, round, or polygonal cells, and the second are fusiform. The latter, elongated elements probably represent stroma of the tumor as Ghelelovitch (43, 44) has demonstrated in cultures. Melanin (54) is deposited quite soon, and the tumors are visible grossly as pigmented spots before the fifth instar. Complete dissolution of cellular components leaves an inert pigmented residue to mark tumor-bearers during pupal and imaginal life. Sections indicate amelanotic tumors to be rare or non-existent, so that scoring in adults is an accurate assessment of tumor incidence in the absence of trauma. The tumors may appear in abdomen, thorax, or head but are most frequently encountered in the abdomen. Occasionally they are situated in the circulatory stream and present a mobility which is striking. Rizki (84) reports that lamellocytes may encapsulate other tissues to produce the appearance of tumors in certain strains. The *tu^h* (75, 76) and *er* (47) tumors also have a different histologic appearance. King, Burnett, and Staley (62, 63) recently have described ovarian tumors in three strains of *Drosophila* bearing respective, female-sterile mutants. Although all tumors studied in our laboratory have been benign, Harnly, El Shatoury (28-30), and Ardashnikov (1) have called tumors malignant which have affected the viability of the individuals bearing them. Russell (85) demonstrated that the lethality of the lethal (1) 7 stock is due to obstruction of the alimentary tract rather than to the presence of the tumor as originally suspected.

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INFLUENCE OF HORMONES ON TUMOR GROWTH

Since the unique regression which has been described is not the usual course of an atypical growth, two types of experiments were performed in order to determine the mechanism for this behavior. Genes which damage the ring gland were introduced into tumor stocks (19), and, in other experiments, larvae were ligated posterior to the ring gland so as to impede access of hormone to the posterior end of larvae (17). In both instances the numbers of tumors were greater when the amount of hormone was diminished. This evidence and that of others (64, 79) supports the hypothesis that the tumor cells are sensitive to metamorphosis hormone and regress in a manner similar to other larval elements. The opposing hormones, ecdysone and neotenin, can be assayed respectively, and ecdysone has been crystallized both from *Drosophila* (20) and *Bombyx* (23, 61). Regression of mammalian as well as insect tumors has been observed when ecdysone is administered, although results so far are neither uniform nor proportional to dosage (23).

INCIDENCE OF TUMORS

A spectrum of tumor strains exists in *Drosophila* (10) with incidence varying from less than 1.0 per cent to more than 99.0 per cent (Table 1). The incidence of tumors in each strain is constant within limits, the variation being wider for a strain such as the *tu g* strain which has intermediate penetrance than for one such as the *tu^{36a}* strain which has low penetrance. The strain with the most constant incidence under the usual conditions in our laboratory has been the

TABLE 1
Incidence of tumors in *Drosophila*

Strain	Times Counted	Months Counted	Number with Tumors	Total No. Counted	Percentage with Tumors		
					Males	Females	Total
<i>bw st tu</i>	7	4	24	4,426	.26	.84	.54
<i>tu^{36a}*</i>	13	8	222	7,473	3.21	2.73	2.97
<i>tu^{36a}</i>	9	5	182	3,394	5.10	5.66	5.36
<i>ed Su²-dx</i>	7	4	385	4,022	11.59	7.52	9.57
<i>f^{257-19B}/In AM</i>	7	4	416	2,449	15.53	17.87	16.99
<i>tu^{wps}</i>	13	8	1,423	8,077	12.10	23.18	17.62
<i>lz³ f</i>	13	8	2,428	10,160	28.53	18.59	23.88
<i>w^{bf} f⁵</i>	7	4	715	2,827	30.20	19.85	25.29
<i>tu^{50d}</i>	15	8	1,901	7,144	29.60	23.31	26.61
<i>bw tu</i>	14	8	2,434	8,614	26.72	29.92	28.26
<i>se e¹¹ tu^{49h}</i>	13	8	3,275	8,799	38.11	36.41	37.22
<i>tu^h*</i>	13	8	2,421	5,464	42.09	46.86	44.31
<i>tu^g*</i>	12	8	2,156	4,626	49.19	43.81	46.61
<i>tu^{48j}</i>	14	8	2,833	5,865	53.66	43.67	48.30
<i>tu^h</i>	13	8	6,616	12,236	50.98	57.69	54.07
<i>vg mt^A bw</i>	14	8	5,944	10,069	56.49	61.09	59.03
<i>y B²⁶³⁻⁴³</i>	7	4	2,274	3,120	69.92	75.77	72.88
<i>tu^g</i>	14	8	9,113	11,967	87.30	65.34	76.15
<i>tu^{48a} vg bw</i>	14	8	10,540	10,555	99.77	99.94	99.86

* Isogenic.

tu^{48a} vg bw strain obtained from Ghelelovitch (38). This inconstant expression of the tumor phenotype in genetically uniform strains is found in mammals as well as *Drosophila* (9).

EFFECT OF ENVIRONMENT ON GENE ACTION

A series of tumor counts was made on eight strains of *Drosophila* over a period of six months under laboratory conditions as nearly uniform as possible with regard to temperature, culture medium, population size, and handling (24). Fluctuations of tumor incidence during this period apparently depended on the specific genes present in each individual stock since the fluctuation in numbers of tumors in the various stocks was neither similar nor predictable. In another series of experiments, isogenic tumor stocks were obtained by appropriate crosses with inversions to prevent crossing over (14). Even though each pair of alleles on the four chromosomes had a common origin, penetrance was not complete. The ambient milieu in conjunction with or without suppressor genes thus may prevent complete expression of the tumor phenotype.

The effect of temperature on tumor incidence is quite striking in some stocks, whereas changes in temperature in others produces little or no effect (Table 1). For example, temperatures ranging from 15 to 30° C. fail to alter the incidence in the *tu^{36a}* stock appreciably. On the other hand, flies of the *tu^{48a} vg bw* strain raised at 30° C. had less than half the incidence of tumors found in the stock when raised at 20° C. and 25° C. respectively. The prolonged life cycle at lower temperatures did not necessarily raise the tumor incidence. Hartung (52) and Harnly, Friedman, Emery, and Glassman (49) found that response to temperature is not uniform for all strains and varies with the tumor genes present. Ghelelovitch (40, 41, 45) extensively investigated the effect of temperature on the *tu^{48a}* strain. Only the maternal effect was changed in the *tu^h* strain when the temperature was varied by Gardner and Woolf (36).

An inverse relationship between the size of the population and tumor incidence has been reported by Herskowitz and Burdette (59) and Wilson (101). This effect is probably related to nutritional state of the larvae. In general, a reduction in the amount of yeast available has resulted in a concomitant diminution in tumor incidence, and certain stages in development are more sensitive to this effect than others, e.g. the third day of larval life in the *tu^{48j}* strain.

TABLE 2
Effect of temperature on incidence of tumors

Tumor Strain	15° C.		20° C.		25° C.		30° C.	
	Percentage Tumors	Total No. Counted	Percentage Tumors	Total No. Counted	Percentage Tumors	Total No. Counted	Percentage Tumors	Total No. Counted
<i>tu^{36a}</i>	1.1	637	1.7	1,091	1.6	1,271	2.7	526
<i>tu^g</i>	90.5	558	99.5	1,281	11.2	1,118	9.5	84
<i>tu^{wps}</i>	20.3	118	14.4	1,122	19.7	1,220	0.0	60
<i>tu^{48j}</i>	72.0	93	95.1	595	82.3	1,113	..	..
<i>vg mt^A bw</i>	74.5	47	93.2	1,347	82.3	1,073	14.6	288
<i>tu^{48a} vg bw</i>	...	...	98.7	1,142	99.9	1,263	42.2	64

In contrast to the results with *Saccharomyces*, Briones and Brncic (5) found that increasing the content of *Torulopsis utilis* in the medium diminished the number of flies with tumors. The reason for divergent results with different yeasts is not clear. Caloric restriction in mammals may also reduce the number of susceptible individuals with tumors as well as prolonging survival (98-100). Extensive work on adding and deleting specific dietary constituents has been done by a number of investigators (33, 34, 39, 42, 49, 57, 72, 74). Prolongation of the larval stage by administration of 2, 4-dinitrophenol delayed the appearance of tumors in experiments of Wilson (102), but contradictory results have been obtained when the effect of various amino acids on the incidence of tumors has been tested (46, 58, 71, 73, 80, 81, 102, 103). The action of vitamins on tumor appearance was inhibited by the appropriate analogue according to Friedmann, Harnly, and Kopac (35).

LOCALIZATION OF TUMOR GENES

The pattern of inheritance of the melanotic tumors is easily followed if susceptibility to tumors is scored rather than the actual incidence of tumors. Exten-

TABLE 3

Chromosomal distribution of genes associated with tumors or focal melanosis in *Drosophila*

I	II	III	IV
<u>l(1)7* l(1)7e*</u>			
<u>l-t*</u>			
<u>l(1)ml*</u>			
<u>l(1)76*</u>			
<u>pg*</u>			
<u>me*</u>			
<u>fu*</u>			
	<u>tu^s</u>		
	<u>tu^{48j}*</u>		
	<u>mt^A*</u>		
	<u>e¹¹tu*</u>		
	<u>tu^{50j}*</u>		
	<u>fes*</u>		
	<u>nw*</u>		
		<u>md*</u>	
		<u>tu^{wps}</u>	
tu ^h	tu ^h	<u>tu^h</u>	
	<u>tu¹</u>	<u>tu¹</u>	
	<u>tu^{49h}</u>	<u>tu^{49h}</u>	
	<u>tu</u>	su-tu	
	su-er	er	
tu ^{36a}	<u>tu^{36a}</u>	tu ^{36a}	
tu ^{49k}	<u>tu^{49k}*</u>	tu ^{49k}	
tu ^w	<u>tu^w</u>	tu ^w	
tu ^{48a} vg bw	<u>tu^{48a} vg bw*</u>		tu ^{48a} vg bw
be(3)	<u>be(3)*</u>	<u>be(3)*</u>	be(3)
tu ²	<u>tu²</u>	<u>tu²</u>	tu ²

Symbols underlined: Main genes for susceptibility.
 Symbols designated*: Localized within chromosome.

sive linkage studies have been reported for mammalian tumor genes, but localization along the chromosome is much more precise in *Drosophila* (Table 3). The number of tumor genes present in a strain may be one or several, and they may be present on one, two, three, or four chromosomes. The contribution of each gene also varies, and both enhancer and suppressor modifiers have been found. The suppressor-erupt-tumor system studied by Glass and Plaine (47) and the modifiers for *tu^h* found in alien strains by Gardner, Scott, and Dearden (37) are good examples. For reasons unknown, many of the main tumor genes are present on the second chromosome. However, only occasionally are alleles found when strains of different origin are compared. An example is the presence of at least one common gene in *tu^{48a} vg bw* and *tu^h* (12) strains. More than one third of the stocks have known modifiers in addition to the main genes, and more than one main gene has been reported for three of them. Both the *benign* tumor studied by Stark and Bridges (93) and the *tu-2* stock studied by Wilson (101) have at least one susceptibility gene present on each of the four chromosomes. Although tumors arise in the presence of a single mutant on either chromosomes I and III, an impressive number of main genes are located on chromosome II. Several of the tumor genes have been localized within narrow limits (Table 4).

TABLE 4
Examples of localized genes for tumor susceptibility

Chromosome I		Chromosome II		Chromosome III	
Tumor	Locus	Tumor	Locus	Tumor	Locus
<u>l(1)7</u>	0.1	<u>fes</u>	5	<u>be³</u>	25
<u>fu</u>	59.5	<u>tu^{48a} vg bw</u>	29.5		
		<u>tu^{48j}</u>	44.3-55.7		
		<u>mt^A</u>	82.0-82.7		
		<u>nw</u>	83.		
		<u>e¹¹ tu</u>	88.		
		<u>tu^{57j}</u>	90.		
		<u>be³</u>	107.		

Recombination of tumor genes has been more difficult to follow than for many visible mutants, but no evidence has been found that tumor pseudoalleles exist. Since the germinal tumor mutants could be either point mutation or associated with chromosomal aberration, it is of interest to determine in this favorable biological material which type of change has taken place in the chromosome leading to the occurrence of tumors. Salivary chromosomes have been examined under both light and electron microscopes, and no evidence of chromosomal aberrations have been found in the region of tumor genes which have been localized by crossing over (16). In certain instances, tumors have appeared in strains with duplications and inversions, but these pre-existing chromosomal aberrations are the only ones which have been observed in the salivary gland preparations. Although this study has not been exhaustive, being confined only to the *tu^{48j}* and *vg mt^A bw* stocks, this evidence is sufficient to show that the tumors may appear without visible chromosomal rearrangement.

Since the time of Boveri (3), great interest has centered around the possible relationship between aneuploidy and cancer. Therefore, the addition of euchromatin and heterochromatin to the normal chromosomal complement to test the effect of changes in gross amounts of chromosomal material within the cell is of interest, and studies have been completed on the effect of additions and deletions of heterochromatic material to cells containing tumor genes in *Drosophila*. This was done by the use of the tandem X:Y chromosome. Through appropriate crosses, the effect of varying multiples of the Y chromosome, which contains little euchromatin in *Drosophila*, was studied. The results indicated that there was no consistent change in tumor incidence, the amount and direction of the change depending on the tumor genes present (24).

MUTATION AND THE ORIGIN OF SUSCEPTIBILITY TO TUMORS

A recurrent idea about the origin of cancer is that a mutation occurs in a somatic cell. Since mutations to tumor susceptibility occur in germ cells, the possibility that this may lead to cancer when it occurs in a somatic cell cannot be denied. However, the event is uncommon in germ cells, and the frequency with which it occurs in somatic cells is open to question. It would seem reasonable to require mutagenic agents to be carcinogenic and carcinogenic agents to be mutagenic should this hypothesis be true. Using the lethal mutation rate on the X chromosome as an indicator, both mutation rate and tumor incidence were determined simultaneously in the *tu^{se}a* strain. When 20-methylcholanthrene was administered as an aerosol, the incidence of tumors was increased in the F₁ generation, but the mutation rate was unchanged significantly (13). On the other hand, nitrogen mustard both augmented mutation rate and tumor incidence (15). The mutation rate in males was increased when formaldehyde was fed to larvae, but the tumor incidence remained unchanged in both sexes (11) in contrast to the sex difference in lethal mutation rate after treatment with formaldehyde. Although negative results are always questionable, the fact that mutation rate and tumor incidence were determined simultaneously and one was increased with an absence of any effect on the other in the case of methylcholanthrene and formaldehyde, casts considerable doubt on the idea that the neoplastic change is very often the result of a somatic mutation.

Should somatic mutation be the sole reason for the appearance of tumors, the germinal mutants which raise tumor incidence would of necessity be mutators. The effect of a mutator was tested in several tumor strains, and no relationship between the presence of mutators and an increase in tumor incidence was found (18). *Florida* stocks were used for these studies, and fluctuations in the mutation rate during the course of experiments made interpretation additionally difficult.

One of the ways in which a neoplastic change could result in an inherited change in somatic cells would be through the loss of a gene or enzyme system. In order to test whether the germinal tumor mutants consist of a simple loss of substance, the *tu^{sa} vg bw* strain was subjected to treatment with both a chemical mutagen, formaldehyde, and X irradiation. Progeny were then tested for reverse mutation toward normal. The experiments with formaldehyde were nega-

tive with no reversions in 34,331 chromosomes tested. However, when the mutation rate was increased approximately threefold by using X irradiation in dosage of 2,000 r, two reversals were found in 76,483 tests. Subsequent crosses suggest these to be true reversions and not due to the appearance of new suppressor mutants. These findings indicate that the structural change which produces a tumor mutant may be different than simple deletion and is reversible.

TUMORS AND GENOIDS

Experiments within recent years on the etiology of leukemia have indicated that a filterable agent is of prime importance in the appearance of the disease (48) despite previous genetic and radiologic studies to the contrary. This has stimulated considerable interest in this facet of the etiology of cancer. The only cytoplasmic particle easily available for study in *Drosophila melanogaster* is the genoid for CO₂ sensitivity (66-68, 82). Chromosomes containing tumor genes were introduced into cytoplasm containing the genoid by genetic methods, using inversions to prevent crossing over, as well as the introduction of the genoid through injections into cytoplasm containing chromosomes carrying tumor genes. The *forte*, *faible*, and *Tr* strains of the genoid were used in these studies. It was found that the presence of genoid in the *tu^{48a} vg bw* strain resulted in a diminished incidence of tumors (21, 22). When loss of sensitivity occurred in some of the individuals used in the investigation, it was found that the lowered tumor incidence remained in the offspring despite the disappearance of sensitivity. Whether the presence of the particle in the cytoplasm alters the expression of tumor mutants in a permanent manner is an interesting question for speculation. Although the plasmagene theory of tumorigenesis has been distinguished chiefly by the absence of experimental data to support the assumption, the utilization of viruses with known effects within the cell in conjunction with known tumor mutants would seem a profitable future avenue of approach, and these results in *Drosophila* indicate positive results will be forthcoming from the combination of genes and genoids.

SUMMARY

Hereditary tumors in *Drosophila* have proved to be a valuable means for investigating some of the factors involved in the origin of tumors, but, even in this relatively simple experimental animal, with brief life cycle and small chromosomal complement, the mechanism is somewhat complicated (Figure 1). The appearance of an atypical growth is naturally modified by the characteristics of the organism in which it is encountered, and *Drosophila* is no exception. The tumors studied are composed of aggregates of hemic cells with related stroma, and their persistence is modified by the hormones controlling metamorphosis. Ecdysone is apparently responsible, at least in part, for their unique regression. Susceptibility mutants, single and multiple, are responsible for the appearance of the hereditary tumors, and penetrance depends on the relative effectiveness of a number of different conditions prevailing during the period of genic action. The same environment change may result in different effects on different genes.

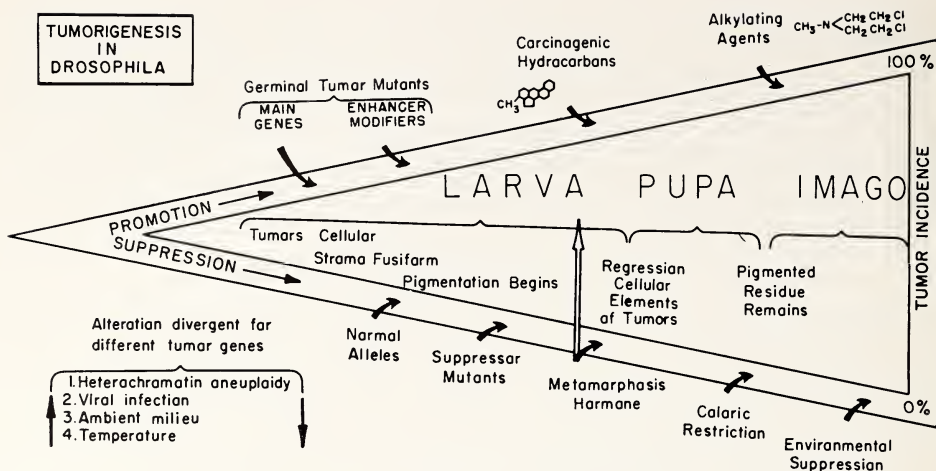


FIG. 1

In general, certain chemicals such as alkylating agents and carcinogenic hydrocarbons may increase the incidence of tumors, whereas caloric restriction and suppressor mutants may reduce the incidence of tumors. No general correlation between agents increasing incidence of tumors and mutation rate has been found, and the germinal mutation to tumor susceptibility is not necessarily a deletion or visible chromosomal aberration. The minute localization and specificity of this chromosomal change in *Drosophila* demonstrated by genetic studies have sufficient prognostic significance for urging refinement of techniques in other organisms in order to discern which intracellular events lead to neoplastic transformation.

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Sex Balance in *Drosophila melanogaster*: Aneuploidy of Short Regions of Chromosome 3, Using the Triploid Method

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The present study is the outcome of previous investigations on sex determination in *Drosophila melanogaster* initiated and carried out by Professor J. T. Patterson and his colleagues and students. Beginning with his work on X-ray induced gynandromorphs, mosaic females, and "aberrant" (i.e., hyperdiploid) males and females, Patterson was interested in learning if a primary female sex factor exists in the X chromosome (Patterson, 1931, Patterson and Stone, 1938). By further studies of hyperdiploid males derived from overlapping X,4 translocations according to a method described by Muller and Stone (1930), the possibility of a major female sex factor was reduced to a short section extending from salivary bands 13A2 to 13A6, the g-pl region (Patterson, Stone, and Bedichek, 1937; Patterson, 1938). Crow (1946) finally ruled out a possible X chromosome primary female sex determiner by synthesizing a diploid male hyperploid for this region. None of the X chromosome hyperdiploid males studied by these investigators was intersexual. An occasional rotation of external genitalia was not taken as a sign of intersexuality in X-hyperdiploid males since this rotation is sometimes found in diploid males hyperploid for a short autosomal region. Conversely, no diploid female hypoploid for a section of the X chromosome displayed any intersexual features. Mickey (1950) briefly described some diploid intersexes arising in an attached-X stock in which detachment had occurred. The intersexes were thought probably to be diploid aneuploids with one X chromosome and a fragment of an X, but it was not possible to make cytological studies.

Meanwhile, Dobzhansky and Schultz (1934) and Pipkin (1940) were able to transform triploid intersexes into weakly functional hypotriploid females by the addition of long fragments of the X chromosome to the 2X3A intersex complement. These investigators agreed that short sections of the X in some cases shifted the sex type of "duplication" intersexes in the female direction. By the overlapping translocation method, Pipkin found that individuals bearing short X chromosome sections, covering in succession the entire chromosome, when added to the 2X3A intersex chromosome set are only intersexual flies. Longer and longer fragments from either the left or right hand end of the X chromosome cause a qualitatively progressive shift in the direction of femaleness until either the right or left hand fragment of two different translocations, 17 (broken between 7F5 and 8B1) and w13 (broken between 9B1 and 9B3), when added to the 2X3A intersex chromosome set, produce weakly functional females (Pipkin, 1940). A shorter left than right hand fragment is required to produce the complete sex shift from intersexuality to femaleness.

The weakly fertile hypotriploid female with the right hand X fragment of X,4 translocation 17 or w13 retained rudiments (one or two prongs) of male sex

combs in some individuals when reared at 23° C. When reared at 18° C, these 17R or w13R plus 2X3A individuals were all sterile, more intersexual in bodily appearance, and developed larger sex combs (up to six prongs). Furthermore hypointersexes lacking a short portion of one of their two X chromosomes, possessing three of each autosome, were shifted strongly in the male direction. Thus triploid aneuploid studies of the X chromosome show that several parts of the X chromosome are concerned with female differentiation and the effects are additive.

Patterson, Brown, and Stone (1940) investigated diploid aneuploidy of the 2nd and 3rd chromosomes. They succeeded in producing hyperdiploid males and females carrying in triplicate each of eight short regions covering successively the entire chromosome 2 and eleven regions, covering all but the leftmost short section, of chromosome 3. Hypodiploid males and females were obtained for five short sections of chromosome 3 but for none of the 2nd chromosome sections. No sex shift was found in either autosomal hyperdiploid females or in the hypodiploid males for five regions of chromosome 3 studied. Burdette (1940) studied a homozygous duplication female bearing a short heterochromatic region just to the left of the centromere of chromosome 3 in quadruplicate, but no change in the sexual differentiation of these females was observed.

With the same 2,4 translocations used by Patterson, Brown, and Stone (1940), Pipkin (1947) studied triploid aneuploidy of chromosome 2 in an effort to determine the role played by this chromosome in sex determination. No shift toward maleness was observed in hyperintersexes (2X3A + fragment of 2) for each of ten short regions covering successively the whole of chromosome 2. Similarly, hypertriploid females (3X3A + fragment of 2) showed no signs of intersexuality. Hypointersexes (2X3A - fragment of 2) were obtained in numbers sufficient for a statistical analysis of the sex types for one region only, and no sex type shift in hypointersexes as compared with control sibling intersexes (2X3A) was found for this region. Hypointersexes for six other short regions and three of the longer regions showed no qualitative shift in a female direction; i.e., they appeared intersexual.

Using the overlapping translocation method, Dubovsky (1939) studied the effect of increasingly longer sections of the left arm of chromosome 3 upon diploid aneuploids. Hypoploids for only one very short region survived. Hyperploids for 5 regions included between 61 and 67B lived, but hyperploids for three longer regions failed to survive. Patterson and Stone (1952) summarized their views on sex determination based on evidence from diploid and triploid aneuploidy.

Two different investigators have described changes in sexual differentiation owing to hypoploidy of the 3rd chromosome. In both cases the deficient regions were minimal in length, and the second case combines hypoploidy with the action of a mutant.

Kelstein (1938, 1939), also using the overlapping translocation method, studied minute duplications and deficiencies (diploid aneuploids) of a section of the right arm of chromosome 3 extending from salivary bands 89A to 94A by means of 18 different 3,4 translocations broken in this short section. The individual duplications and deficiencies studied extend only a few salivary bands in length and are thus much shorter than those studied by Patterson, Brown, and

Stone (1940) in diploid aneuploids and by Pipkin (1947 and this paper) in triploid aneuploids. Kelstein described hypoploids for the section from 89D to 90A which were female in appearance but possessed male sex combs (the size of the sex combs was not given). Hypodiploid males for this region were described as invariably having rotated genitalia. No intermediate intersexual forms were described (i.e., there was no mention of fragmentation of the male genitalia or change in anal plates or of forms with abnormal female genitalia). The author does not make it clear if the female-like intersexes were 1X or 2X. No cytological examinations were described, and no dissections were made to study the internal genital tract. Kelstein lists 130 hypoploid males and 163 hypoploid females for this region (XV), but he does not enumerate the number of female-like intersexes. No doubt is here cast upon the results as reported especially since other aneuploid data are in agreement with what has been described by other investigators.

Intersexuality has also been described as resulting from the combination of diploid hypoploidy with a mutant. Goldschmidt (1948, 1949a, 1949b, 1949c) found low-grade intersexuality in males carrying the 3 chromosome dominant mutant *Beaded* together with any one of several different minutes (tiny deficiencies) which could be located on either the 2nd or 3rd chromosomes. Neither *Beaded* nor the minute alone produced intersexual effects. The *Beaded-Minute* intersexes were interpreted by Sturtevant (1949) as representing incomplete development of the anal and genital imaginal discs. On the other hand, Goldschmidt (1949c) presented drawings and descriptions of the internal genitalia of the *Bd-Mn* intersexes showing what looks very like seminal receptacles and in one specimen, a ventral receptacle and rudimentary vagina. He also pointed out that a reduction of the genitalia and anal plates is likewise characteristic of type III triploid intersexes. Partly from his work on the *Beaded-Minute* intersexes, Goldschmidt (1955) concludes that "sex determination is actually a function of intercalary heterochromatin, a generalized function, not capable of being dissolved into sets of multiple genes with special actions," since *Beaded* is a heterochromatic mutant and the minutes are deficiencies in heterochromatic regions.

A number of autosomal mutants causing intersexuality in diploid (2X2A) females but usually not affecting 1X2A males occur in several species of *Drosophila*. A recessive mutant in both the 2nd chromosome (the *ix* mutant, Morgan, 1943, and its allele, *ix*², Meyer, 1959) and in the 3rd chromosome (the *tra* mutant, Sturtevant, 1945, as well as the dominant allele of *tra* called *Hr*, Fung and Gowen, 1956, 1957) have been described in *D. melanogaster*. Sturtevant (1920) found a recessive intersex mutant in the 2nd chromosome of *D. simulans*. Lebedeff (1939) studied a recessive mutant, *ix*, in *D. virilis*, and Newby (1942), a dominant mutant, *Ix*^B, not allelic with Lebedeff's mutant, in the same species. Dobzhansky and Spassky (1941) reported such a recessive mutant in *D. pseudoobscura*, and, similarly, Hollingsworth (1953) and Spurway and Haldane (1954) in *D. subobscura*.

Species hybrids in *Drosophila* which yield intersexes have been described in the *repleta* group. Cases summarized by Patterson and Stone, 1952, include those of Wharton, 1940; Sturtevant, 1946; and Ward and Stone, 1952. In Sturtevant's case, an autosomal gene in *D. neorepleta* was found, capable when present in one

dose in a female hybrid between this species and *D. repleta* of conditioning her eggs in a male direction so that the resulting zygotes with two *repleta* X chromosomes developed into intersexes.

Bridges' (1921, 1922) discovery of triploid intersexes in *D. melanogaster* possessing two X chromosomes and three of each autosome led him to assign the male determining genes to the autosomes. Dobzhansky and Bridges (1928) ruled out a connection of the tiny fourth chromosome with sex determination since it could be present in single, double, or triple dose in diploids, and its dosage did not affect the sex type of triploid intersexes. The absence of a Y chromosome likewise was long ago found not to affect the sexual differentiation in XO non-disjunctive males though these are sterile (Bridges, 1922). Dobzhansky and Schultz (1934) concluded that the Y chromosome does not influence the sex type of triploid intersexes since the mean sex type of Y 2X3A intersexes does not differ significantly from that of sibling 2X3A intersexes in the material studied by them (Dobzhansky and Schultz, 1934).

As in *D. melanogaster*, autosomes must play a male-determining role in other species of *Drosophila*. Triploid intersexes similar to those found in *D. melanogaster* have been reported in *D. americana* (where motile sperm are found in the extreme male type) (Stalker, 1942); *D. simulans* (Neuhaus, 1939); as hybrids between *D. melanogaster* and *D. simulans* (Schultz and Dobzhansky, 1933); *D. pseudoobscura* and as hybrids between *D. pseudoobscura* and *D. miranda* (Dobzhansky and Spassky, 1941).

The purpose of the present investigation is to complete the triploid aneuploid studies of the large autosomes by studying sex balance in the 3rd chromosome.

MATERIALS AND METHODS

The 3,4 translocation stocks used in the present experiments with the salivary map points of breakage in chromosome 3 include the following: T(3,4) e (Dobzhansky, 1929; Lewis, 1956), broken at 79E; T(3,4) c (Dobzhansky, 1929; Lewis, 1951), broken at 86C; T_A(3,4) 13 (Patterson, *et al.*, 1934; Patterson, Brown, and Stone, 1940), broken at 67E; T_A(3,4) 12 (Patterson, *et al.*, 1934; Patterson, Brown, and Stone, 1940), broken at 73C; T_A(3,4) 2 (Patterson, *et al.*, 1934; Patterson, Brown, and Stone, 1940), broken at 94A3-4; T_A(3,4) 28 (Patterson, *et al.*, 1934; Patterson, Brown, and Stone, 1940; Lewis, personal communication), broken at 94D3-4; T_A(3,4) 30 (Patterson, *et al.*, 1934; Patterson, Brown, and Stone, 1940), broken at 96E; T(3,4) 85C (Lewis, personal communication), broken at 85C; T(3,4) 89E (Lewis, personal communication), broken at 89E; T(3,4) H7, broken at 66C; T(3,4) H1, broken in the chromocenter; T(3,4) H3 broken in the chromocenter; T(3,4) H5, broken at 96E; and T(3,4) H6, broken at 98A. The T(3,4) H stocks were recently produced by X-rays at Howard University and their points of breakage here determined for the first time. The older T_A(3,4) stocks were checked anew in salivaries by the author to be sure that they were the original stocks as labeled.

The triploid stock developed for these studies had free X chromosomes and contained no known chromosomal abnormalities. The recessive mutant γ^2 (yellow body with black bristles) marked the X chromosome; *ru* (roughoid), the left end

of chromosome 3; and *ca* (claret), the right end of chromosome 3. The character *ru* proved to give no difficulties in classification since it is not only far more pronounced in its disturbance of facets in 3A flies than in 2A flies, but also the entire eye is markedly narrowed in triploids and intersexes whereas it is only slightly narrowed in most diploid flies. The homozygous γ^2 ; *ru ca* triploid stock was rather weak, though not highly inbred. It had to be made up twice during the course of the experiments, and it was necessary to use 40 triploid females to one bottle in experimental crosses in order to keep down mold infections.

All of the experimental crosses developed in a B.O.D. incubator; most of the work being done at $22^\circ \pm 0.5^\circ$ C. Some special crosses were carried out at $26^\circ \pm 0.5^\circ$ C. Pans of water were kept in the B.O.D. incubator to keep the relative humidity about 75–80%.

To study aneuploids for either the right or left end of the 3rd chromosome, males carrying a 3,4 translocation broken near an end of 3 and bearing the normal alleles of *ru* and *ca* in the broken 3rd chromosome, also heterozygous for an intact 3rd chromosome bearing the marker mutants *ru* and *ca* were crossed with homozygous γ^2 ; *ru ca* triploid females. A diagram illustrating this type of experimental cross appears in Figure 1. For example, in the case of a translocation with a break at the right end of chromosome 3, hyperintersexes appear either γ^2 ; *ru* or simply *ru*. Members of the first group, appearing γ^2 ; *ru*, receive their 2 γ^2 X chromosomes from the triploid mother, a Y chromosome from the translocation male parent, and possess three intact *ru ca* chromosomes plus the right hand fragment carrying the normal allele of *ca*. The second group of intersexes, appearing *ru*, is similar to first with respect to 3rd chromosome content but such flies possess one wild-type X chromosome derived from the translocation male parent and one γ^2 X chromosome from the triploid mother. Similarly, hyperintersexes bearing the left hand fragment of a translocation broken near the left end of the 3rd chromosome appear γ^2 ; *ca* or simply *ca*. Hypertriploid females carrying a right hand end fragment in excess of 3X3A appear *ru*; hypotriploid females lacking one dose of this region from the 3X3A complement appear *ca*. Conversely, hypertriploid females carrying a left hand end fragment in excess of 3X3A appear *ca*; hypotriploid females lacking one dose of this region from the 3X3A complement appear *ru*. The genetic composition of one group of hyperintersexes and hypertriploid females involving a right hand end region is diagrammed in Figure 2.

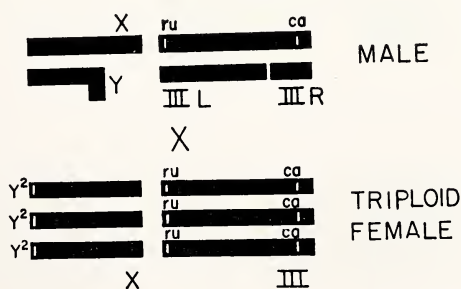


FIG. 1. Experimental cross designed to produce aneuploids involving end region of chromosome 3.

Aneuploids for interior regions of chromosome 3 were obtained by the overlapping translocation method. Homozygous $\gamma^2; ru\ ca$ triploid females were crossed with males heterozygous for two different 3,4 translocations with neighboring points of breakage. One translocation carried the recessive marker mutant ru and the normal allele of ca ; the second translocation, with point of breakage to the left of the former, carried the recessive marker mutant ca and the normal allele of ru . Such a cross is diagramed in Figure 3. Hyperintersexes appearing in the progeny are either $ru\ ca$ or $\gamma^2; ru\ ca$, depending on the source of the X chromosomes. Similarly, the hypointersexes are either wild-type or γ^2 . Hypertriploid females appear $ru\ ca$; hypotriploid females, wild-type. The genetic composition of one group of hyperintersexes and hypointersexes, and also that of hypertriploid females appear in Figure 4.

In some cases the male parents carried ru in the 3,4 translocation broken to the left and ca in the 3,4 translocation broken to the right. Here the hyperintersexes appeared γ^2 or wild-type; the hypointersexes, $\gamma^2; ru\ ca$ or $ru\ ca$; hypertriploid females, wild-type; hypotriploid females, $ru\ ca$.

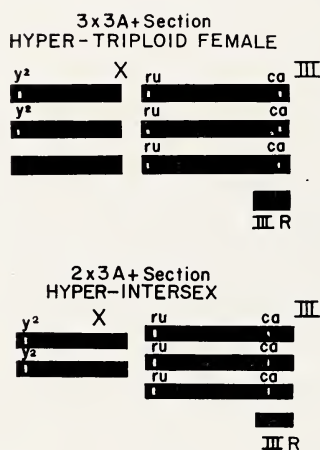


FIG. 2. Genetic composition of (1) a hypertriploid female carrying an end region of chromosome 3 in excess of 3X3A and (2) of one group of hyperintersexes for an end region.

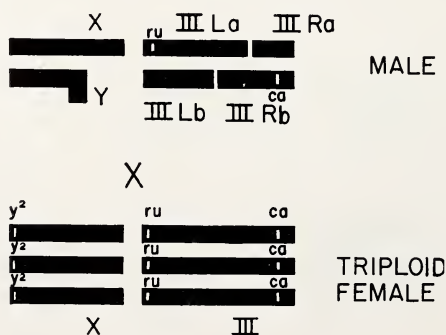


FIG. 3. Experimental cross designed to produce aneuploids involving an interior region of chromosome 3.

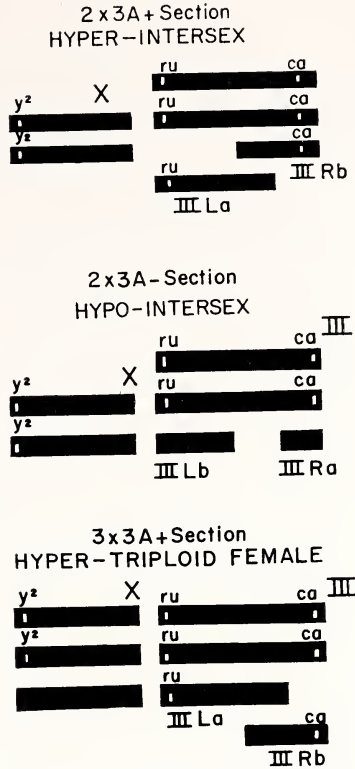


FIG. 4. Genetic composition of (1) one group of hyperintersexes, (2) one group of hypointersexes, and (3) a hypertriploid female involving an interior region of chromosome 3.

Hyper- or hypoploidy of the diploid progeny of y^2 ; $ru \quad ca$ triploids and males heterozygous for one or two different translocations was determined similarly, according to the recessive marker mutant(s) displayed. Diploidy was distinguished from triploidy (or the 2X3A intersex condition) by the wing texture which is fine in diploids and the eye facets which are smaller in diploids than the eye facets of the 3A forms.

Separate counts were made from each bottle of an experimental cross so that homogeneity tests could be made to determine if genetic modifiers were sufficiently variable in the triploid or translocation stocks to shift the sex types of control intersexes (2X3A) hatching in different bottles of the same experimental cross, the temperature being kept constant.

EXPERIMENTAL RESULTS

1. Survival of 3rd chromosome aneuploids.

Survival of 3rd chromosome aneuploids for short regions is summarized in the diagram presented in Figure 5. The top line in Figure 5 gives the salivary map location of the points of breakage of the 3,4 translocations used in the study. The stock numbers of these 3,4 translocations are placed near the bottom line in Figure 5. Survival of hyperintersexes, hypertriploid females, and hyperdiploid

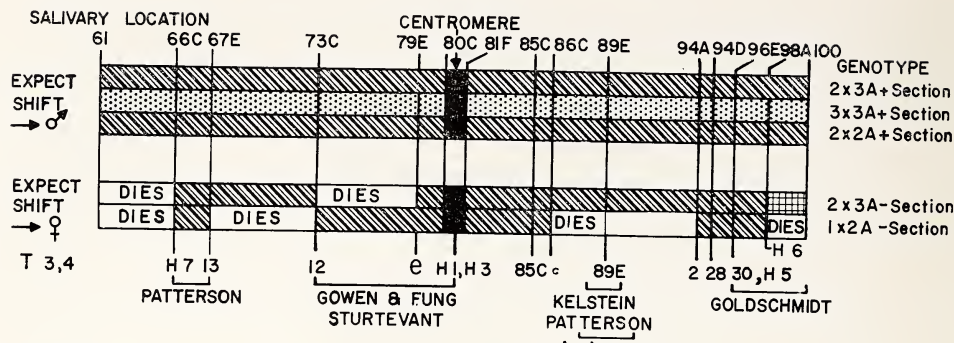


Fig. 5. Diagram summarizing survival of various 3rd chromosome aneuploids.

females, respectively, for each short region, is indicated in the upper section of Figure 5. Survival of hypointersexes and hypodiploid males is indicated in the lower section of Figure 5. If a single region possessed strong male-determining potency, the phenotype of the former group would be expected to be shifted in a male direction. Similarly, the phenotype of the latter group would be expected to be shifted in a female direction. According to Figure 5, both hyperdiploid females and hypertriploid females survived for each region studied. None showed any intersexual features. Hyperintersexes survived for each region with the possible exception of the region between $T_A(3,4)$ 12 and the chromocenter, a point to be discussed later. In all cases hyperintersexes and surviving hypointersexes appeared typically intersexual. According to the lower section of Figure 5, hypointersexes and hypodiploid males for the left hand end region and hypodiploid males for the right hand end region did not live. Further, hypointersexes for the interior region 12-e and hypodiploid males for interior regions 13-12, possibly 12-chromocenter, 89E-2, and c-89E failed to survive. It should be noted that the region 30-H6 was not directly tested owing to the difficulty of introducing the marker *ca* into $T_A(3,4)$ 30. Instead this section is represented in Figure 5 as yielding hypodiploid males and hypointersexes because hypodiploid males and hypointersexes for the longer region 2-H6 survive, as may be determined from Table 1. Only one specimen of a hypointersex for region H6R was found. A statistical analysis of the sex types of hyperintersexes and hypointersexes, in cases where these survived in large enough number will be considered in a latter section.

Table 1 presents the data upon which Figure 5 is based. The first column contains the genetic composition of the male parents carrying one or two 3,4 translocations, with the translocation broken to the left appearing first. The 3rd chromosome marker mutants displayed by the aneuploids can be determined by examining the disposition of *ru* and *ca* in the male parent. For example, the 40 hyperdiploid male progeny of $\gamma^2; ru\ ca$ triploids and *ca*,13/*ru*,12 males appeared $\gamma^2; ru\ ca$; 55 hyperdiploid females for this region were *ru\ ca*; and 208 hyperdiploid females were $\gamma^2; ru\ ca$. A phenotypic appearance of the X chromosome marker mutant γ^2 is indicated within Table 1; a phenotypic appearance of the 3rd chromosome marker mutants *ru* and *ca* must be deduced from the genetic

composition of the male parents. In the second column of Table 5 appears the region tested and the temperature (22° C or 26° C) at which the experiments were conducted. The salivary map limits of each region may be ascertained from Figure 5. One group of control intersexes (2X3A) is included in the last column of Table 1. The control intersexes chosen to appear in Table 1 appeared wild-type in the case of experiments designed to study end regions of chromosome 3; *ca*, in the case of nearly all of the experiments designed to study interior regions of chromosome 3. The γ^2 and γ^2 ; *ca* control intersexes in these two respective types of experimental crosses do not occur with as great a frequency as the wild-type or *ca* control intersexes owing to the greater frequency of 1X2A eggs over 2X2A eggs from the triploid mother. Table 1 is not intended to give information for quantitative comparisons of sex types of aneuploid intersexes vs. the control intersexes, but to show the total number of the various kinds of aneuploid individuals observed.

The evidence for survival of hypodiploid males for the region 12-H3 rests on 12 γ^2 ; *ru ca* males listed in Table 1. Only one such γ^2 ; *ru ca* "hypoploid" male for the similar region 12-H1 was found and none for the region 12-e. H3 is known to be triplo-4, and the presence of an extra chromosome 4 may have favored survival of 12-H3 hypodiploid males. On the other hand, these γ^2 ; *ru ca* males listed as hypodiploids for region 12-H3 might have been derived from non-disjunction of all parts of both 3,4 translocations in the male parent, resulting in a sperm with no part of chromosome 3. Upon union of such a sperm carrying a Y chromosome, with an egg containing one γ^2 X chromosome and two *ru ca* 3rd chromosomes, viable γ^2 ; *ru ca* males could result. Such non-disjunctional forms have occurred rarely in the progeny of γ^2 ; *ru ca* triploids and males heterozygous for two different 3,4 translocations with the breakage points rather far apart, to be discussed in a later paper. The 12 γ^2 ; *ru ca* males listed in Table 1 as hypodiploid males for region 12-H3 did not show extreme signs of aneuploidy although some of the corresponding γ^2 ; *ru ca* hypodiploid female sibs had reduced eyes, facets fused, outstretched wings, certain bristles missing and vaginal plates protruding. On the other hand, one γ^2 ; *ru ca* male listed as hypoploid for 12-H3 showed a slight reduction in number of sex comb prongs on one leg. The author is therefore uncertain whether these γ^2 ; *ru ca* males and their γ^2 ; *ru ca* and *ru ca* female sibs are hypoploids as Figure 5 indicates, or whether they represent forms owing to non-disjunction of both 3,4 translocated chromosomes in the male parent. If future work shows that these flies listed as hypodiploids are due to non-disjunction, some doubt is cast on the 4 *wild-type* and 1 γ flies listed in Table 1 as hyperintersexes for region 12-H3.

Both control and aneuploid intersexes listed in Table 1 were classified according to five sex types originally described by Dobzhansky (1930) and since used in several investigations of triploid intersexes (Dobzhansky and Schultz, 1934; Pipkin, 1940, 1947). In the present material only 5 groups were found, ranging from extreme male type I to female type V possessing normal vaginal plates, female type anal plates, female abdominal coloration, and male sex combs. The extreme female type VI, lacking sex combs was absent. Examination of the control intersexes in Table 1 shows that the largest female group was type IV, composed of intersexes with external genitalia and anal plates of the female type

TABLE 2
Homogeneity tests of sex type frequencies of hyperintersexes with those of one group of control (2X3A) intersexes

Region	Control Intersexes					Total	Hypointersexes					Total	Chi Square	f	P	Hom. or Het.
	I	II	III	IV	V		I	II	III	IV	V					
H7 L (22°C)	97	33	12	22	0	164	30	7	0	1	0	38	7.899	3	<0.05	Het.
13-12 (22°C)	93	41	25	20	0	179	15	14	5	7	0	41	4.314	3	0.3>P>0.2	Hom.
H1-85C (26°C)	119	78	26	34	0	257	105	52	14	23	0	194	3.053	3	0.5>P>0.3	Hom.
85C-c (22°C)	65	31	17	29	0	142	63	37	16	15	0	131	4.613	3	0.5>P>0.3	Hom.
c-89E (22°C)	180	63	18	32	3	296	68	10	10	0	0	88	19.022	4	<0.05	Het.
89E-28 (22°C)	93	53	70	81	0	297	10	11	12	2	1	36	17.582	4	<0.05	Het.
2-28 (22°C)	41	38	31	30	4	144	74	42	22	36	4	178	8.248	4	0.1>P>0.05	Hom.
28-30 (22°C)	70	24	14	28	1	137	21	8	7	2	0	38	6.244	4	0.2>P>0.1	Hom.
28-30 (26°C)	58	52	20	42	0	172	25	6	4	2	0	37	16.186	3	<0.05	Het.
2 R (22°C)	209	106	64	68	2	449	39	8	2	0	0	49	21.805	4	<0.05	Het.
30 R (22°C)	133	86	27	94	4	344	119	15	20	4	0	158	80.522	4	<0.05	Het.
H5 R (22°C)	91	31	18	31	1	172	37	2	3	0	0	42	19.032	4	<0.05	Het.
H6 R (22°C)	182	39	24	33	2	280	24	3	4	0	0	31	4.029	4	0.5>P>0.3	Hom.

but often protruding, misplaced, and sometimes imperfect, male or intermediate abdominal coloration, and male sex combs. Arbitrarily included in this type IV class were occasional individuals which showed both vaginal plates and a fragment of male external genital apparatus. For example, among the 61 type IV wild-type control intersexes occurring in the cross H5/ *ru ca* males with γ^2 ; *ru ca* triploid females, 6 displayed mixtures of both male and female genitalia.

All of the aneuploid intersexes in Table 1 appeared typically intersexual. Since there were no obvious shifts in the male or female direction among the hyper- and hypointersexes, chi square homogeneity tests (using the method of Brandt and Snedecor, described by Fisher, 1932) were made to see if the intersex sex types were distributed with the same frequency in aneuploid intersexes as in one group of their respective control (2X3A) intersexes. To be sure that any shift in sex type found in aneuploid intersexes as compared with their 2X3A sibling control intersexes is owing to a dosage change of the short 3rd chromosome section involved in the aneuploidy rather than to genetic modifiers present in the triploid or translocation stocks which varied from bottle to bottle of the same experimental cross, the following precautions were taken: Chi square homogeneity tests were made to determine if the sex type frequency of one group of control intersexes varied from bottle to bottle of the same experimental cross. Counts from bottles yielding fewer than five intersexes of the chosen control group were discarded. In most cases the sex type frequencies of control intersexes coming from different bottles of the same experimental cross were homogeneous. Where sex type frequencies of control intersexes coming from different bottles of the same experimental cross were homogeneous, these were pooled. Counts from the occasional bottles giving sex types of the chosen control intersex group heterogeneous with the frequencies of sex types of control intersexes of other bottles of the same experimental cross were discarded from the calculations. The number of such discarded intersexes may be deduced by comparing frequencies appearing in Table 1 with those for the same experimental cross in Tables 2 and 4. In the case of wild-type control intersexes occurring in the progeny of H5/ *ru ca* males with γ^2 ; *ru ca* triploid females, counts from a sizable number of bottles had to be discarded owing to heterogeneity (presumably due to genetic modifiers) but this was an unusual situation.

Finally, sex type frequencies of hyperintersexes (or hypointersexes) were compared with the sex type frequencies of their respective sibling control intersexes. Pooled counts of both aneuploid and control intersexes were used only from bottles in which the control intersex sex type frequencies had proven homogeneous. Table 2 gives chi square homogeneity tests comparing pooled sex type frequencies of one group of control intersexes and those of hyperintersexes derived from the same experimental cross for each of 12 short regions in cases where hyperintersexes were numerous enough for statistical studies. P indicates the probability that the two sets of sex type frequencies differ owing to chance alone; $P < 0.05$ being taken as indicating heterogeneity. The number of degrees of freedom, i.e., one less than the number of sex type groups is represented by "f" in Table 2.

According to Table 2, sex type frequencies of hyperintersexes from the left

hand end region H7L, the interior region c-89E, and three right hand end regions; 2 R, 30 R (at 22° C), and H5 R; proved heterogeneous with the sex type frequencies of their respective control intersexes. In each case, a slight shift toward more male-like sex types occurred among the hyperintersexes as compared with their respective control intersexes. A very slight shift in the female direction occurred among the hyperintersexes for the interior region 89E-28 as compared with the control intersexes. Homogeneity existed between sex type frequencies of hyperintersexes and control intersexes for one right end region, H6R, and five interior regions; 13-12, H1-85C; 85C-c; 2-28, and 28-30 (22° C). The latter region, 28-30, forms a part of 2 R since $T_A(3,4)$ 2 and $T_A(3,4)$ 28 are broken very close to one another at 94A and 94D respectively. The heterogeneity between sex type frequencies of hyperintersexes for region 28-30 and those of their control intersexes at 26° C is due to a change in the distribution of the sex types of the control intersexes in the female direction in the 26° C series compared with the 22° C series. The distribution of sex types of hyperintersexes for region 28-30 in the two temperature series is seen to be closely similar.

To indicate the magnitude of sex type shift, means of sex types of those hyperintersexes and their respective control intersexes for regions in which the two sets of frequencies were heterogeneous are compared in Table 3. According to the last column, the standard deviation of the difference between means of control and corresponding hyperintersexes is significant except for the last region, 89E-28.

For comparison with the small sex type shifts in the male direction found in the 3rd chromosome hyperintersexes, it was of interest to determine if a shift in the female direction would be produced in hyperintersexes for a short X fragment against the particular genetic background of the γ^2 ; *ru ca* triploid stock of present experiment. Males hyperplod for a minute X chromosome fragment including the normal alleles of *yellow* and *achaete* plus a tiny right hand portion including the normal allele of *bobbed* were crossed to γ^2 ; *ru ca* triploids. (The males were of the genetic constitution $Y^s.X \text{ In EN } y.Y^L sc^s y^+$.) The gray intersex progeny carried 2 complete X chromosomes plus the very short X fragment, a Y chromosome, and 3A. The *yellow*² intersexes possessed only 2X3A. From this cross the gray hyperintersexes were found with the following sex type frequencies: 52 I, 47 II, 43 III, 50 IV, and 1 V. *Yellow* control intersexes occurred as follows: 119 I, 41 II, 11 III, and 22 IV. The two sets of sex type frequencies were heterogeneous with a chi square of 56.681. The gray hyperintersexes had a

TABLE 3

Comparison of mean sex types of hyperintersexes and control intersexes

Region	Mean, Control Intersexes	Mean, Hyperintersexes	Difference Between Means	S.D. of Difference
H7 L (22°C)	1.750	1.253	0.487	0.127
2 R (22°C)	1.993	1.245	0.748	0.091
30 R (22°C)	2.273	1.424	0.849	0.093
H5 R (22°C)	1.953	1.191	0.762	0.123
c-89E (22°C)	1.699	1.341	0.358	0.094
89F-28 (22°C)	2.468	2.167	0.301	0.160

mean sex type of 2.484 compared with a mean of 1.672 for *yellow*² control intersexes. The difference between the means was 0.812 ± 0.122 , representing a significant shift in the female direction in the hyperintersexes carrying the minute X chromosome fragment in excess of 2X3A. These results were to be expected in view of the work of Dobzhansky and Schultz (1934) with a minute X fragment covering a similar X chromosome region.

Homogeneity tests of the sex type frequencies of control intersexes and hypointersexes for seven short regions of chromosome 3 are presented in Table 4. Four of these regions, 2-30, 28-30, 28-H5, and 2-H6, cover much of the same salivary map region. Homogeneity between sex type frequencies of hypointersexes and their control intersexes exists in all of the cases except region 2-H6 and region 28-30 (at 26° C). The mean sex type of region 2-H6 hypointersexes was 1.225; that of the control intersexes, 1.673; the difference between the means of 0.488 ± 0.137 being barely significant. Table 4 shows that the distribution of sex type frequencies of region 28-30 hypointersexes of the 22° C series does not differ significantly from that of the control intersexes, but at 26° C hypointersexes for this region have a sex type mean of 1.877 compared with 2.267 for control intersexes. The difference between the latter means is 0.390 ± 0.141 , barely significant. In the cases of region 2-H6 and 28-30 (at 26° C), the slight sex type shift of hypointersexes as compared with control intersexes is in the male direction.

Since aneuploid intersexes were characterized by late hatching, an investigation was made of the hatching time of 2X3A intersexes of the γ^2 ; *ru ca* stock classified according to their sex type, to determine if there is a difference in time of hatching (or in survival) of male-like intersexes vs. female-like intersexes. The experiment was patterned after that of Dobzhansky (1930), but intersexes were classified according to sex types. All eggs were deposited during a two hour interval. Development took place at $22^\circ \text{C} \pm 0.5^\circ \text{C}$. The sex type frequencies of early hatching intersexes on the 12th day after egg laying was 59 I, 33 II, 15 III, 16 IV, and 1 V. Intersexes hatching late on the 16, 17, 18, 19, and 20th days include the following: 17 I, 10 II, 5 III, and 4 IV. The sex type frequencies of early hatching intersexes were homogeneous with those of late hatching intersexes with a chi square of 0.145 and $0.8 > P > 0.7$.

None of the hypertriploid females carrying in excess of 3X3A short sections of chromosome 3 showed any characteristics of intersexuality. No obvious phenotypic changes were found in hypertriploids for the following sections: H7 L, H1-85C, 85C-c, 89E-28, 89-E, 28-30, 2-28, e-H1, e-12, or H1-H3. (H1-H3 hyper- and hypotriploids could not be distinguished as such since both breaks are in the chromocenter, but both groups of aneuploids looked like normal 3X3A triploids.) Body size was slightly reduced in hypertriploids for 12-13, 12-H1, and 2-30. Wings were held slightly apart and up in hypertriploids for all of the right hand end regions 2 R, 30 R, H5 R, and 6 R. In H7-13 and also in c-89E hypertriploids the body size was smaller than that of a diploid female; eyes were reduced; bristles, thin; legs misshapen; and wings were held apart. Hypertriploids carrying 13 L in excess of 3X3A had very large, round, flat eyes with facets profoundly disturbed, and vaginal hairs were often missing. Hypertriploids for the following regions were found to be weakly fertile: 13-12; c-89E; 2 R; 30 R; H5 R; H1-85C; e-H1.

TABLE 4
Homogeneity tests of sex type frequencies of hypointersexes with those of one group of control (2X3A) intersexes

Region	Control Intersexes					Total	Hypointersexes					Total	Chi Square	f	P	Hom. or Het.
	I	II	III	IV	V		I	II	III	IV	V					
2-30 (26°C)	100	95	22	23	1	241	79	40	11	11	0	141	8.583	4	0.1>P>0.05	Hom.
28-30 (22°C)	70	24	14	28	1	137	17	9	7	4	0	37	4.432	4	0.5>P>0.3	Hom.
28-30 (26°C)	58	52	20	42	0	172	35	31	5	10	0	81	7.995	3	<0.05	Het.
28-H5 (26°C)	24	18	7	12	0	61	50	38	8	13	0	109	3.078	3	0.5>P>0.3	Hom.
2-28 (22°C)	41	38	31	30	3	143	37	32	24	25	2	120	2.571	4	0.7>P>0.5	Hom.
2-H6 (22°C)	67	22	11	10	0	110	34	4	1	1	0	40	8.019	3	<0.05	Het.
85C-c (22°C)	65	31	17	29	0	142	53	33	16	31	0	133	1.091	3	0.8>P>0.7	Hom.
H7-13 (22°C)	79	37	4	7	0	127	45	14	2	2	0	63	1.783	3	0.7>P>0.5	Hom.

Phenotypic changes are in general more pronounced in hypotriploid females lacking a short section of 3 from the 3X3A complement than in hypertriploid females. Nevertheless, hypotriploid females for short regions H7-13; 13-12, c-89E, 28-30, 28-H5; e-H1 appeared like normal triploids. Eyes were reduced in size; ocelli sometimes missing; wings outstretched; bristles thin; legs misshapen in hypotriploids for regions H7 L, 13 L, 89E-28, and H1-12. Hypotriploids for right hand end regions 2 R, 30 R, H5 R, and 28 R had wings sometimes clipped medially and eye facets and wing texture intermediate between diploid and triploid size. Wing texture was also intermediate between diploid and triploid texture in hypotriploids for 6 R. A similar syndrome of phenotypic change was sometimes seen in various diploid and triploid aneuploids for the same section, as, for example, holding the wings slightly apart in hypertriploid females, hyperdiploid females, and hyperintersexes for right hand end regions or the very large round eye and dark trident pattern in hypertriploid females, hyperdiploid females, hyperdiploid males, and hyperintersexes for H7 L.

A usually small counterclockwise rotation of male genitalia was found occasionally in both hyperdiploid and hypodiploid males aneuploid for short sections of chromosome 3. Among hyperdiploid males the numbers with rotated and normal genitalia were as follows: H7 L, 5 rotated, 76 normal; 13 L, 6 rotated, 35 normal; 13-12, 1 rotated, 39 normal; 85C-c, 1 rotated, 86 normal; 89E-28, 1 rotated, 95 normal; 30 R (22° C) 2 rotated, 101 normal; 6 R (22° C), 1 rotated, 72 normal. Among hypodiploid males the numbers with rotated and normal genitalia were as follows: 85C-c, 2 rotated, 7 normal; H7-13, 8 rotated, 46 normal. Genitalia were occasionally protruding in H7-13 hypodiploid males. The region H7-13 in Figure 5 is marked by "Patterson" to indicate that Patterson, Brown, and Stone (1940) found hypodiploid males with occasional rotated and sometimes imperfect genitalia in hypodiploid males for almost this same section. Rotation of genitalia was also found in one out of 6 super males that were hypoploid for region 28-30. One male hypoploid for region 2-30 and one male hyperploid for 6 R showed lack of anal plates and genitalia.

DISCUSSION

Although Bridges (1939) found that individuals with 3X4A as well as 2X3A were intersexual, the present work on chromosome 3 and that of Pipkin (1947) on chromosome 2 show that no single short autosomal region is responsible for the shift toward intersexuality in triploid intersexes. No hypertriploid (3X3A plus a short section) for any region of chromosome 3 (present work) or chromosome 2 (Pipkin, 1947) showed any signs of intersexuality. The normal allele of the *Hr* and *tra* mutants (Fung and Gowen, 1956, 1957; Sturtevant 1945) is located either in the region 12-e or e-H1, as Figure 5 indicates. There is therefore no evidence from triploid aneuploid experiments that this normal allele functions as a male determiner. It may be, of course, a female determiner as Stone (1942) suggested possible.

Small shifts in a male direction were found in the present experiment in hyperintersexes for several short regions of chromosome 3 but for none of chromosome 2 (Pipkin, 1947). Three slightly different right hand end regions of chromosome

3 produced the largest shifts in a male direction in hyperintersexes. These shifts are comparable in size to those produced in a female direction by the addition of a very short section of the X chromosome to the 2X3A intersex complement. On the other hand, none of 7 different hypointersexes lacking a short section of 3 from the 2X3A complement showed a shift in the female direction. The sex types of one region, 2-H6 even showed a barely significant shift in the male direction when compared with the sex types of 2X3A sibling control intersexes. This last region forms a part of the right hand end region 2 R which in hyperintersexes causes a definite shift in the male direction. Hypointersexes for two short sections of the X chromosome, in contrast, were shown by Pipkin (1940) to be strongly shifted in the male direction.

The small shifts in a male direction in hyperintersexes for certain sections of the 3rd chromosome may then be interpreted in one of three ways: (1) as sex balance shifts owing to dosage changes of the particular aneuploid region involved; (2) as owing to retardation of development of these late hatching hyperintersexes, assuming that triploid intersexes begin development as males, followed by a turning point to female development (Dobzhansky, 1930); (3) as owing to possible differential survival on the part of male-like hyperintersexes as compared with female-like hyperintersexes for the particular regions involved. In connection with this last possibility the experiment of time of development of the members of different intersex sex type groups carried out in the present investigation shows that among ordinary 2X3A stock intersexes the sex distribution of early hatching 2X3A intersexes differs by chance alone from the sex type distribution of late hatching intersexes of the same age but subject to the unfavorable environment of an older culture.

Hypodiploid males for several regions showed occasional rotation of genitalia, an observation also made by Patterson, Brown, and Stone (1940). This rotation is not, contrary to Kelstein (1938, 1939), taken as a sign of intersexuality because occasional rotation also was found in hyperdiploid males for several regions. The rotation is thought to be the consequence of genic imbalance and does not represent a sex shift. Patterson, Brown, and Stone (1940) report no intersexuality in hypodiploid males for a longer region which includes the region XV studied by Kelstein (see Figure 5).

Third chromosome aneuploid studies of the present investigation as well as those of the 2nd chromosome (Patterson, Brown, and Stone, 1940; Pipkin, 1947) and X chromosome (Dobzhansky and Schultz, 1934; Pipkin, 1940) support the conclusion that dosage changes of portions of the X chromosome are far more effective than dosage changes of portions of either of the large autosomes in affecting sex balance.

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SUMMARY

A study of triploid and diploid aneuploids occurring in the progeny of males heterozygous for one or two different 3,4 translocations and triploids failed to reveal any marked shifts in sexual differentiation owing to dosage change of the portion of chromosome 3 involved in the aneuploidy. Hypertriploid females with no signs of intersexuality survived for each of 14 short regions which covered the entire chromosome 3. The mean sex type of hyperintersexes for 5 short regions, 3 of which include the right hand end of chromosome 3, were significantly more male-like than the mean sex type of corresponding sibling control intersexes with 2X3A only. No sex type shift in the female direction was found among the hypointersexes for 7 different short regions. Further there was no qualitative shift in sex differentiation among hyper- and hypointersexes occurring too seldom for statistical analysis of their sex types. An occasional rotation of genitalia was found in both hyperdiploid and hypodiploid males for several regions. It was not taken as an indication of intersexuality.

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Gene-Environment Interaction in Relation to the Nutrition and Growth of *Drosophila*

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INTRODUCTION

In former studies of the genetic variation of body size in *Drosophila melanogaster*, it has been the practice to supply cultures with excess food since this minimises environmental variance when the ordinary live yeast cultures are used. However, under natural conditions, or the usual competitive regime in laboratory bottle or population cage, such conditions would be rather exceptional. Indeed there is little doubt that variation in the quantity and quality of the food supply comprises the major part of the environmental variation encountered by growing larvae. The characteristic genetic attributes of populations will be established under sub-optimal conditions and hence analyses which are confined to the most favourable environment cannot detect gene-environment interactions which may be highly relevant to the properties of intrapopulation variance. Therefore, gene-environment interactions which are associated with different kinds of sub-optimal diet are being studied in some detail and I shall describe briefly some of the first results of this work.

The approach depends on the use of chemically defined, aseptic media upon which the larvae are grown. Various workers have contributed to the development of such media including Tatum (1939), Schultz, St. Lawrence and Newmeyer (1946), Begg and Robertson (1950), but the later refinements are due to Sang (1956) who has determined the quantitative requirements which permit growth to the normal size and at the same rate as on the usual live yeast medium. If the concentration of one or more essential nutrient is inadequate the length of the larval period is increased and body size may be reduced as well, according to the nature and severity of the deficiency. The agar gel medium includes 7 B vitamins, together with casein as amino-acid source, ribonucleic acid, cholesterol, lecithin, fructose and salts, so there are many ways in which a diet can be made inadequate. Most relevant, however, will be the suboptimal diets which reproduce the sort of nutritional conditions commonly encountered under competitive conditions. *Drosophila* lives on yeast and, from the published analyses of yeast it has been inferred (Sang, in press), that the various B vitamins, with the possible exception of folic acid, are unlikely to be limiting factors since they are abundant in relation to minimal requirements. Hence variation in the availability of other essential constituents is probably more important and, in particular, variation in the protein level suggests an obvious starting point. The data described here consist of comparisons of the performance of different strains when grown on the usual live yeast medium, on media deficient in casein but otherwise supplied with excess of other constituents and also on a medium in which the optimum ratio of constituents is maintained but the concentration

in the agar gel is reduced. The adverse effects of the latter medium are probably chiefly due to protein deficiency. The synthetic medium which has been modified in this way is that referred to as Medium C (Sang 1956).

GENERAL OBSERVATIONS

Before giving the results a few general observations are necessary. Body size is expressed as three times the natural logarithm of thorax length, measured in 1/100mm., since this provides an adequate index of weight. Rough estimates of percentage differences in weight can be made by multiplying observed differences between means by 100; this enables the relative magnitude of effects to be kept in perspective.

The length of the larval period, where recorded, is measured in days converted to natural logs, and is based on the egg to adult period minus the duration of the pupal period which is comparatively constant and remarkably independent of both the duration of the larval period and differences in adult body size due to selection.

A lengthening of the larval period constitutes the first sign that a larval diet is inadequate. Especially with protein deficiency there may be a considerable lengthening of larval life without reduction of final size, but of course, with more drastic shortage, body size is reduced as well. There is, therefore, a definite ability to regulate body size when protein is a limiting factor and this can be regarded as a form of homeostasis. Figure 1 shows the sort of relation which exists between body size and length of the larval period with variable protein levels. The two wild populations compared evidently differ in their ability to regulate in this way. If the protein level falls too low, body size declines and progressive reduction of body size is associated with a corresponding proportional increase in development time. For a precise comparison of strains with respect to the protein concentration at which the regulation breaks down, a range of comparisons is needed. But for a quick survey it is sufficient to provide a diet which reduces body size appreciably and then strains can be compared in terms of how far body size is reduced below the level attained under favourable conditions.

Genetic differences in this homeostatic regulation of body size are relevant to fitness generally. With respect to the general competitive advantage of quick versus slow development time there is little doubt. With regard to adult body size it is known that a sparse larval diet which reduces body size also causes a lower rate of egg production. In particular, it has been shown (Robertson 1957) that a given reduction in body size is associated with a proportional reduction in the number of ovarioles, without affecting their individual rate of production, and hence a proportional decline in egg production. Therefore differences in percentage reduction of body size below the maximum probably account for the major share of what is commonly called fitness with respect to the specific unfavourable conditions.

Finally, in these tests which measure the effects of sub-optimal diets, the nutrient gel is homogeneous and always in excess and so the results are due to differences in the chemical composition of the diet and competition between larvae may be disregarded.

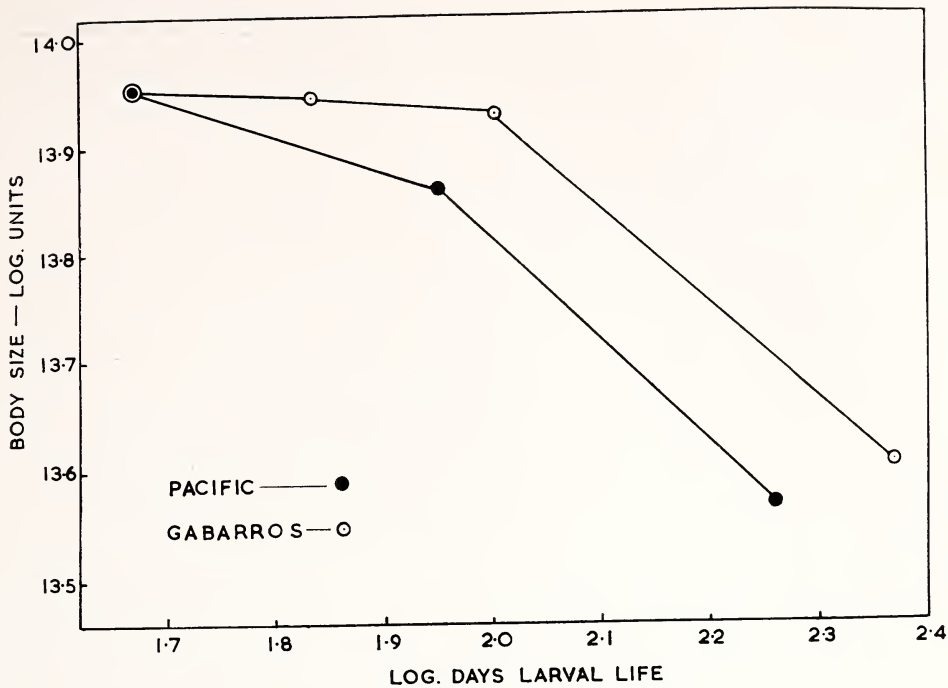


FIG. 1. Body size and length of larval period on synthetic media which differ only in casein concentration.

EXPERIMENTAL RESULTS

These comprise the following: In two different wild populations of *Drosophila melanogaster*, Kaduna and Pacific, mass selection for large and small size has been carried out for 5 generations, after which the performance of the selected and unselected strains were compared on different media. The Kaduna population was selected for wing length, which is highly correlated with body size, by Mr. Barry Latter for other purposes and I have to thank him for kindly providing me with subcultures for testing. In the Pacific population selection was for thorax length; two large and two small strains were selected simultaneously and the comparisons included crosses between large and small strains as well. In addition the performance of a number of inbred lines has been compared with the population from which they were derived.

(a) *The Kaduna population.* To provide a suitable index of deviation of the selected strains from the mean size of the unselected population, the differences between means of flies reared on the usual live yeast medium is expressed in terms of the within-culture standard deviation of the unselected flies. Thus the selected strains deviate some $1.5-2\sigma$ —equivalent to 8–10% difference in body weight. The relation between deviation from mean and response to various sub-optimal diets is shown in Fig. 2, in which the ordinate measures the decline in body size below maximum size. Four treatments were used, two levels of protein shortage and two levels of nutrient concentration. The points represent the average for 20–30 individuals drawn from several replicate cultures.

The results are very striking since individuals from both the selected strains

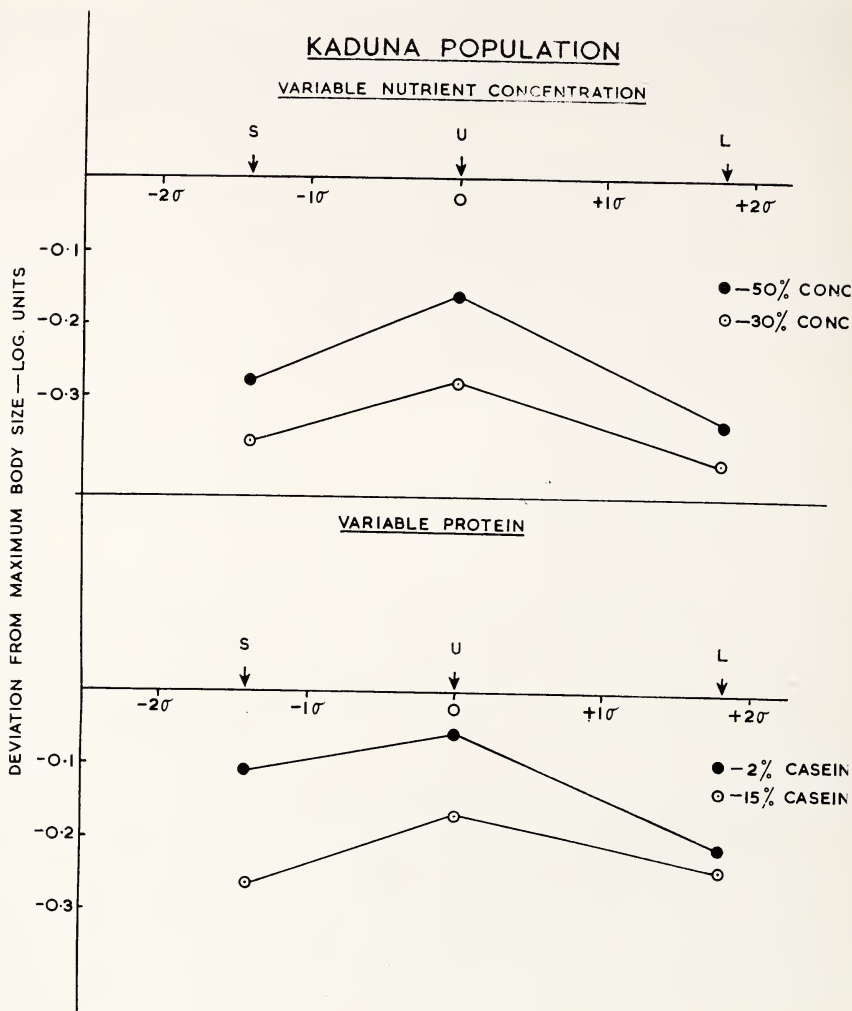


FIG. 2. The relation between deviation from the mean body size of the unselected population under favourable conditions and decline in body size on media deficient in protein or with reduced concentration of nutrients. The deviation is measured in terms of the within-culture standard deviation of the unselected flies reared under the favourable conditions of the usual live yeast medium.

suffer a greater decline in body size than those from the unselected population. Thus a protein deficiency which results in about 5-6% decline in body size in the unselected populations, causes 10% and 20% decline in the small and large strains respectively, while a reduction of nutrient concentration which reduces the size of the unselected flies by about 20% results in a 30% decline in the selected strains. Thus deviation from the mean in either direction lowered the ability to regulate body size on these sub-optimal diets.

An additional point of interest relates to the within-culture variance. It is a familiar experience to find that this increases when flies are grown on sub-optimal media. In view of the homogeneous nature of the medium and the fact

that about 70% of the variance of body size, under optimum conditions, is genetic (Robertson 1957) there is little doubt that the greater variance represents genetic differences in ability to cope with the adverse environment. This phenomenon is shown in Table 1, which deals with the pooled within-culture

TABLE 1
Within-culture variance of body size (Kaduna)

Treatment	Unselected		Large		Small	
	σ^2	d.f.	σ^2	d.f.	σ^2	d.f.
OPTIMUM	0.0035	28	0.0027	28	0.0043	28
2% Casein	0.0061	25	0.0150	24	0.0109	28
1.5% Casein	0.0055	33	0.0045	6	0.0147	33
50% Concentration	0.0043	24	0.0059	22	0.0073	19
30% Concentration	0.0074	25	0.0051	30	0.0046	15

variance of the different series and treatments. Variance on the sub-optimal diets is consistently higher than the corresponding variances on live yeast media and there is also some indication, especially in the small strain, that the relative increase in variance is greater in the selected than the unselected populations.

(b) *The Pacific population.* The results of a similar comparison of the Pacific strains on the usual medium and on 50% nutrient concentration are shown in Figure 3; the four possible crosses between a large and small strain are also shown. Here the two small strains are rather similar in size, while the large strains differ, one (AL) being only about 0.5σ away from the mean. The results of these comparisons follow essentially the same pattern as the Kaduna comparisons; the regression of decline in size on squared deviation from the mean of the unselected is clearly significant ($b = -0.25 \pm 0.008$). However, some additional points must be noted. Firstly, although the general trend is clear enough there is evidently variation with respect to the deviation from the mean and relative decline with this treatment. Thus the small A strain is apparently no worse than the unselected population in these terms and the same is true of one of the large strains (AL). Secondly, the crosses deviate appreciably from the intermediate value, as usual in such tests, and all exceed the unselected population in body size. Although they differ slightly in percentage decline, their average is about the same as that of the unselected flies. Thus, although the large and small parent strains may have lower ability to maintain body size on this sort of deficient diet, the more normal sized F_1 reacquires the properties of the unselected population.

Taken together then, the results of the Kaduna and Pacific tests indicate that selection for large and small body size under favourable conditions of excess food, results in genetic changes which lower the animal's ability to cope with sub-optimal, especially protein deficient, diets. This means that the selected individuals grown in such media take relatively longer to reach maturity and will lay fewer eggs than the unselected flies, even though their egg production may not differ from that of the unselected population when all are grown under

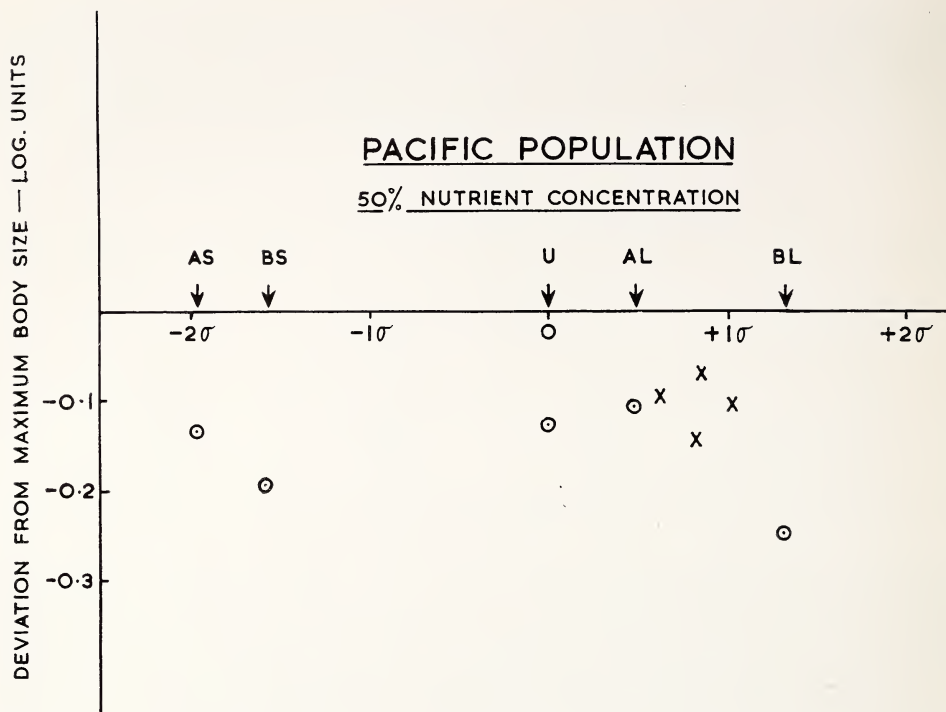


FIG. 3. The relation between deviation from the mean and response to a medium with low nutrient concentration; the crosses refer to the F_1 of matings between a large and a small strain.

favourable conditions, as is usually the case when such selected strains are compared (Robertson 1957). Thus with selection under favourable conditions, the effective loss of fitness will be greatly influenced by environmental, especially nutritional conditions.

The characteristic relationship revealed in these tests might, *à priori*, be regarded as peculiarly characteristic of selection for large and small size as opposed to selection for some other character or it might be regarded as a less specific by-product of a general loss of balance or co-adaptation whenever the original genetic equilibrium is disturbed. It might be argued, for instance, that selection has led to an increase in the level of inbreeding and that this accounts for the lower performance under adverse conditions. To check this point, a similar comparison has been made between the wild Pacific population and 4 highly inbred lines derived from this population. If non-specific effects are involved such lines should be subject to a greater decline than the unselected flies and might even be expected to be a good deal worse than the selected strains. In practice it turns out however that they resemble the unselected flies in response to diets deficient in protein or with a reduced concentration of nutrients. Table 2 shows that with a lower concentration of nutrients the averages for the inbred lines and the wild stock are almost identical; for the low casein diet the results are more variable, and line 10 is missing due to an accident during sterilisation of the eggs, but the average decline is close to that of the wild flies and one line, No. 4, suffers less decline than the former. It will be noted that the media lead to

TABLE 2

Performance of inbred lines and the wild stock on favourable and adverse diets

Genotype		Body Size on Live Medium (Log Units)	Decline in body size	
			50% Concentration	Protein Deficient
Inbred line	3	14.044±.008	—0.393	—0.300
	4	14.053±.008	—0.402	—0.162
	6	13.981±.008	—0.450	—0.271
	10	13.963±.008	—0.372	
			Average	—0.244
Wild population		14.113±.011	—0.408	—0.219

The means are based on the measurement of about 30 flies shown equally for 3-4 replicated cultures.

a relatively greater decline in body size than in the other tests. This may be partly due to differences in the live medium or the temperature between experiments, but is probably mainly due to a relatively higher temperature of autoclaving since certain amino-acids tend to be inactivated when autoclaved in the presence of sugar (Evans and Butts 1949). This circumstance, however, does not affect the validity of the results and it is difficult to avoid the conclusion that the characteristic behaviour of the selected strains is directly due to change in the frequency of genes which affect body size under optimal conditions. It will be noted that the inbred lines are smaller than flies of the wild population, some 7 to 15% smaller, which is quite typical of the effects of inbreeding in this species. It has been concluded, on other grounds (Robertson and Reeve 1955), that such a decline due to inbreeding is qualitatively different in physiological origin and genetic behaviour from a comparable decline due to selection and the contrast in reaction to deficient diets of inbred lines and selected strains—deviating about equally from the base population—supports that view.

DISCUSSION

The leads given by these experiments are being followed up and discussion of their wider implications will be deferred until various current tests are completed. However, the present data are relevant to two familiar problems which have been encountered in earlier studies relating to body size, namely, the asymmetry of selection response and the stability of average body size. With respect to the former, mass selection for large and small body size leads to different rates of response in the two directions. Selection for small body size generally proceeds further and faster away from the base population than selection for large size which eventually reaches a point at which the response to selection stops, although plenty of genetic variation remains and it is easy to select down again (Robertson and Reeve, 1952; Robertson, 1955). Now the relative deviation from the original population of strains selected for large and small size depends on the larval diet. Under favourable conditions a large and small strain may deviate about equally from the parent population but when the comparisons are made on say, a protein deficient medium, the difference between the large and unselected strains diminishes and may even be oblit-

erated, whereas the deviation of the small strain increases, so that a more or less symmetrical situation is converted into a highly asymmetrical one in terms of deviation from the unselected population. This appears very clearly in Table 3; the corresponding deviations have been averaged for different treat-

TABLE 3
Deviation of body size from the mean of the unselected population—log units

Treatment	Kaduna		Pacific	
	Large	Small	Large	Small
Normal	0.12	—0.09	0.09	—0.07
Sub-optimal	0.02	—0.21	0.02	—0.09

Based on average of values for different sub-optimal treatments (Kaduna) or strains (Pacific).

ments (Kaduna) or for both large or small strains (Pacific). Hence it is likely that progressive change in the gene arrays, due to selection for large body size, may result in more exacting demands on the available diet, which, looked at the other way, becomes effectively sub-optimal and so this sort of gene-environment interaction could contribute to the eventual barrier to selection progress. If this is true, the actual level at which the barrier or plateau is established will be greatly influenced by the composition of the diet provided during selection. Also, if the comparisons between the inbred lines and the wild population are typical, loss of heterozygosity could be quite unimportant in the development of such a situation. Naturally with selection for small size, comparable changes will assist rather than hinder the progress of selection.

It is well known that the average body size of individual wild populations maintained in the laboratory remains comparatively stable over long periods (Reeve 1954). In unpublished experiments it was found that when samples from an initially heterogeneous population were run for many generations under competitive conditions, the mean body size of the replicated populations was remarkably similar when compared under the usual favourable conditions. It is reasonable to assume that the stability of the mean reflects an underlying genetic equilibrium such that deviation either way tends to lower fitness, but the manner in which this might be effected has remained obscure. Under favourable conditions, the genetic variance of body size is largely additive in behaviour, it is easy to select either way and the changes produced by a few generations of selection do not afford much evidence of loss of fitness as judged by records of egg production, viability and so forth. But the present experiments reveal an entirely different picture when the larval diet is sufficiently inadequate to reduce body size below the maximum level. It is reasonable to infer that progressive deviation from the mean—measured in terms of body size under optimal conditions—will be correlated, on protein deficient diets, with relatively greater decline in body size, a longer larval period and a lower rate of egg production, which adds up to loss of fitness.

Naturally it has to be shown that the sort of adverse conditions provided in these experiments are relevant to natural conditions and this is being checked.

But in the light of existing information about the nutrition of *Drosophila* and the inter-relations which exist between metabolic processes, it would be rather surprising if they were not.

SUMMARY

There is a well marked capacity for regulating body size in *Drosophila*, to be regarded as a form of homeostasis. Sub-optimal diets frequently cause a lengthening of the larval period but no decline in body size, unless the nutritional deficiency becomes too severe. Genetic differences in the ability to maintain body size have been demonstrated in the performance of strains, selected for a few generations for large and small size, with that of the unselected base population on chemically defined, aseptic media, deficient especially in protein. Performance is measured in terms of the relative decline in body size on such media compared with the size attained under optimal conditions provided by the usual live yeast medium. There is good evidence that individuals of the selected strains are inferior to the members of the unselected population in this respect. Comparisons between highly inbred lines and the parent population show that the former are, on average, no worse than the outbred population, and hence the characteristic relation between deviation from the mean of the wild population and decline in body size, under sub-optimal conditions, is peculiarly characteristic of selection for large and small body size and cannot be attributed to less specific effects such as inbreeding. The results are discussed in relation to the asymmetry of the response to selection for large and small body size, the level at which the response to selection for large size ceases, the stability of body size in populations and the relevance of such gene-environment interactions to natural conditions.

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Observations on Variegated Position Effects in *Drosophila melanogaster*¹

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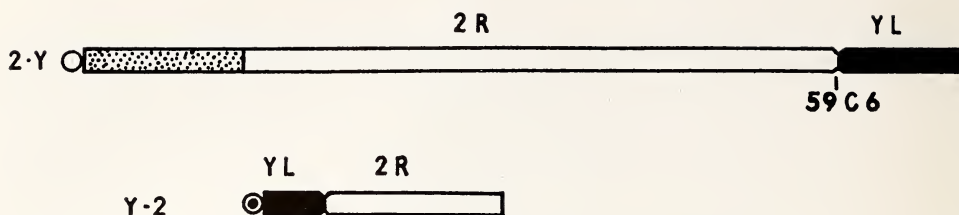
Variegated eye color traits in *Drosophila* have been known since the early days of radiation genetics (Glass, 1933 and 1934) and numerous investigators have demonstrated that both the brown-variegated eye colors and the white-mottled mutants result from chromosome breakage and rearrangement of segments such that euchromatin is brought into contact with heterochromatin or that heterochromatic segments are transposed or translocated to a position adjacent to euchromatin (Baker, 1952 and 1953; Hannah, 1951; Hinton, 1949; Lewis, 1950; Mickey, 1954; Schultz, 1936; and Slatis, 1955a and 1955b). In other words, the irregular distribution of pigment in the eyes which resulted in the mottled or spotted distribution was actually a position effect. One case of particular interest is described in this paper and an analysis is given of its behavior under varying conditions.

This chromosomal aberration, No. 10C-2, arose in a male germ cell which had been irradiated with gamma rays from a Cobalt-60 source.² Adult wild type males of the Oregon-R strain were irradiated and only those germ cells were used which were spermatids or mature sperms at the time of treatment. The aberration produced a dominant phenotypic variegation of eye pigment which varied under different genetic combinations. The mottling was more conspicuous in males than in females.

Genetic tests showed that stock No. 10C-2 contained a reciprocal translocation between the long arm of the Y chromosome and the right arm of the second chromosome, the break in the latter being very close to the *brown* locus. A preliminary report of this translocation has been published (Mickey, 1955). A diagram of this rearrangement is shown in figure 1 and a camera lucida drawing of a metaphase figure from a ganglion cell appears in figure 2. Cytological analysis of salivary gland chromosomes reveals the break in 2R to be at 59C6 on Bridges map (Bridges, 1935). The error in identification is not more than one or two bands. Figures 3 and 4 show photomicrographs of the salivary gland chromosomes in a structural heterozygote; figure 3 shows the Y.2 chromosome and figure 4, the end of the 2.Y chromosome.

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² Grateful acknowledgment is given to Dr. Howard Vogel for aid in treating the experimental flies with gamma rays from a Cobalt-60 source at Argonne National Laboratory.



10C-2 STOCK

FIG. 1. Diagram showing the segmental interchange between the right arm of the second chromosome and the long arm of the Y chromosome in the 10C-2 stock.

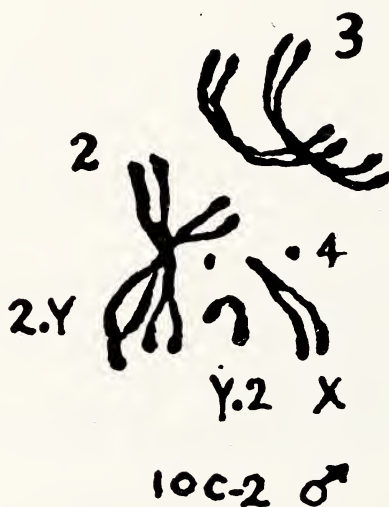
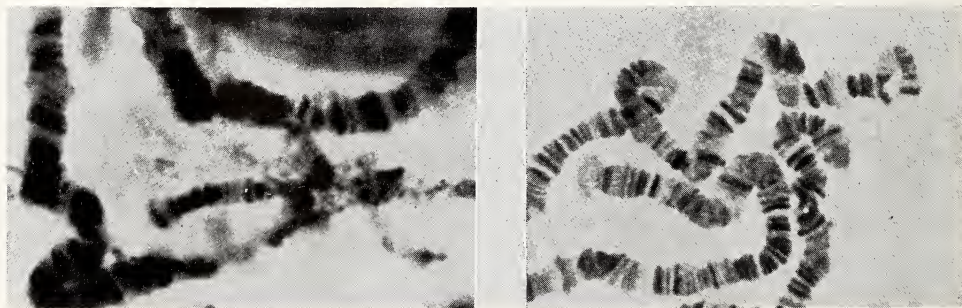


FIG. 2. Camera lucida drawing of a metaphase figure from a ganglion cell showing the 2.Y and Y.2 chromosomes.



FIGS. 3 and 4. Photomicrographs of salivary gland chromosomes showing (left) the Y.2 chromosome protruding from the chromocenter, and (right) the 2.Y chromosome and the tip of the normal second chromosome bent around to make terminal adhesion with the heterochromatic Y segment.

VARIEGATED MALES

Males of the 10C-2 stock have a chromosome complement of $X/Y.2; 2/2.Y; 3/3; 4/4$. The eye color is distinctly variegated or mottled with brown spots on wild type red background or, in an individual homozygous for *vermilion*, brown flecks on a *vermilion* background. Such males are fertile since both parts of the Y chromosome are present when the individual is heterozygous for normal 2R. Both parts of 2R are present also, one on the Y.2 chromosome and the other on the normal second. Random distribution of the 2.Y and normal 2 would be expected to produce half the males heterozygous for 2.Y and carrying the Y.2 chromosome and the other half homozygous for the normal second chromosome but also carrying the Y.2, thus being deficient for part of the Y but having a duplication for the tip of 2R. The former is just like the 10C-2 parent male, whereas the latter carries a duplication of 2R and is sterile because of a deficiency for part of the Y chromosome. Furthermore, the latter males might be expected to be non-mottled if both 2.Y and Y.2 chromosomes are required for the production of variegation, or perhaps more likely, to be only slightly mottled because of the reduced amount of heterochromatin present.

In the cross of
10C-2 males ($X/Y.2; 2/2.Y$) x wild type females ($X/X; 2/2$)
normal segregation can be diagrammed as follows:

♂	♀ X;2	
$X;2$	$X/X; 2/2$	= normal wild type female
$X;2.Y$	$X/X; 2/2.Y$	= deficiency for tip of 2R; lethal
$Y.2;2$	$X/Y.2; 2/2$	= wild type male; deficiency for part of Y; duplication for tip of 2R; sterile.
$Y.2;2.Y$	$X/Y.2; 2/2.Y$	= 10c-2 mottled male

Thus all females derived from this cross would be expected to be wild type, whereas half of the males would be variegated and the other half, wild type if the duplication for the tip of 2R survived; but there would be a skewed sex ratio with approximately twice as many males as females. Genetic crosses confirmed the prediction made on the above assumptions.

A test was set up to determine whether the 2.Y chromosome could survive heterozygously without Y.2 by crossing 10C-2 males which were heterozygous for *nubbin*³ wings to attached-X females carrying *yellow* and *forked* on the two

³ *Nubbin* is a second chromosomal recessive gene located at approximately 43 on the crossover map. It results in very small wings which are thin, with entire margins and venation essentially unchanged, but with a tendency to curve upward or downward. The halteres are somewhat reduced. (Mickey, D.I.S.—23).

X chromosomes and homozygous for *nubbin* on the second chromosomes as follows: $X/Y.2; 2(nub)/2.Y$ males x $y/Y.2(nub)/2(nub)$ females from which F_1 males of two classes were expected:

$X/Y; 2.Y/2(nub)$ and $X/Y; 2(nub)/2(nub)$,

thus all non-*nubbin* males should be $2.Y/2(nub)$ and therefore should be deficient for the tip of 2R (that is, barring non-disjunction of X and Y.2). The actual crosses produced all *nubbin* males; no non-*nubbin* males survived. These

genetic results were confirmed by cytological observations. Salivary gland preparations showed the condition of Y.2 without 2.Y to be present, but the reverse was never observed; in other words, the presence of 2R in triplicate was viable but in the hemizygous condition it was lethal.

Another test was devised to check the viability of combinations deficient for the tip of 2R, which involved crossing 10C-2 males with females homozygous for *speck* on the second chromosome, as follows:

$X/Y.2;2(+)/2.Y$ males $\times$ $X/X;2(sp)/2(sp)$ females

which should give rise to four types of zygotes:

1. $X/Y.2;2(sp)/2(+)$ = wild type male; sterile because deficient for part of Y; duplication of tip of 2R
2. $X/Y.2;2(sp)/2.Y$ = 10C-2 mottled male
3. $X/X;2(sp)/2(+)$ = wild type female
4. $X/X;2(sp)/2.Y$ = lethal because deficient for tip of 2R.

If class 4 flies were viable then females showing *speck* should have appeared, since the gene would have been hemizygous with no wild type allele to cover it. No such females were recovered, which indicated that the loss of the end of 2R reproduced lethality. Again, twice as many males as females were expected if type 1 males, which carried a duplication for the tip of 2R were viable. One half of the males should have been variegated and the other half should have been wild type. The cross actually yielded a one to one ratio.

From the above cross the F_1 wild type females (type no. 3) were mated individually to 10C-2 males which were heterozygous for *speck*:

$\text{♀ } X/X;2(sp)/2(+)$ $\times$ $\text{♂ } X/Y.2;2(sp)/2.Y$.

The zygotes produced by this cross should have been as follows:

- a. $X/X;2(sp)/2(sp)$ = *speck* female
- b. $X/X;2(sp)/2.Y$ = lethal because deficient for tip of 2R
- c. $X/X;2(sp)/2(+)$ = wild type female
- d. $X/X;2(+)/2.Y$ = lethal because deficient for tip of 2R
- e. $X/Y.2;2(sp)/2(sp)$ = wild type male; sterile because deficient for part of Y; duplication of tip of 2R.
- f. $X/Y.2;2(sp)/2.Y$ = 10C-2 male.
- g. $X/Y.2;2(+)/2(sp)$ = wild type male; sterile because deficient for part of Y; duplication of tip of 2R.
- h. $X/Y.2;2(+)/2.Y$ = 10C-2 male.

Therefore the ratio expected among females was one *speck* to one wild type, and such a ratio actually was recovered. Among males the expected ratio of one wild type to one variegated male was obtained.

When class 2 males were crossed with *speck* virgins, the following results were obtained:

Cross: $\text{♂ } X/Y.2;2(sp)/2.Y$ $\times$ $\text{♀ } X/X;2(sp)/2(sp)$

- Progeny:
- a. $X/X;2(sp)/2(sp)$ = *speck* female
 - b. $X/X;2(sp)/2.Y$ = lethal; deficiency 2R
 - c. $X/Y.2;2(sp)/2(sp)$ = wild type male; duplication 2R; sterile
 - d. $X/Y.2;2(sp)/2.Y$ = 10C-2 male.

The females were all *speck* and non-variegated, whereas the ratio among males was one wild type to one variegated; but the sex ratio was skewed again, being two males to one female.

Still another genetic test indicated that the 2.Y chromosome without the Y.2 produced an inviable combination. When 10C-2 males were crossed with females heterozygous for the dominant markers *Curly* and *Plum* the following results were obtained:

Cross:	δ X/Y.2;2(+)/2.Y x ϕ X/X;Cy/Pm	
Progeny:	a. X/X;Cy/+	= <i>Curly</i> females
	b. X/X;Pm/+	= <i>Plum</i> females
	c. X/X;Cy/2.Y	= lethal
	d. X/X;Pm/2.Y	= lethal
	e. X/Y.2;Cy/+	= duplication tip 2R; sterile, deficient for part of Y
	f. X/Y.2;Pm/+	= duplication tip 2R; sterile, deficient for part of Y
	g. X/Y.2;Cy/2.Y	= <i>Curly</i> mottled males
	h. X/Y.2;Pm/2.Y	= <i>Plum</i> mottled males

Since no variegated females appeared and since both *Curly* mottled and *Plum* mottled males occurred, the prediction was borne out.

VARIEGATED FEMALES

Variegated females occasionally appeared in crosses of 10C-2 males with wild type females as a consequence of non-disjunction; but when 10C-2 males were crossed with virgin females from an attached-X stock, variegated females regularly appeared. This was possible since the attached-X females carried a Y chromosome and those females receiving both Y.2 and 2.Y could survive and produce mottling of eye color. The ratio obtained was one variegated female to one wild type female, but all males were wild type.

NON-DISJUNCTION

Non-disjunction occasionally occurs involving the X chromosome and the Y.2 chromosome thus producing a female with variegated eyes. In crosses between 10C-2 males and wild type females normal segregation produced a ratio of one variegated male to one wild type female as diagrammed earlier. The non-disjunction zygotes would be:

1. X; 2/2.Y = lethal; deficient for 2R and most of Y chromosome
2. X; 2/2 = wild type male; sterile because the Y is missing.
3. X/X/Y.2; 2/2 = wild type female carrying Y.2 but not 2.Y and therefore not variegated; but also duplication for tip of 2R.
4. X/X/Y.2; 2/2.Y = variegated female carrying both Y.2 and 2.Y

This last type is a variegated female who was produced through primary non-disjunction and whose germ cells would give rise to secondary non-disjunction.

From a cross of 10C-2 male with a variegated attached-X female a few solid brown-eyed males and females were obtained. Presumably such individuals

could arise by non-disjunction giving genotypes of $X/Y.2/Y.2$; $2.Y/2.Y$ for the males and $XX/Y.2/Y.2$; $2.Y/2.Y$ for the females, which are homozygous for both $Y.2$ and $2.Y$ chromosomes. These may be termed homozygous translocations or structural homozygotes. The rate of non-disjunction in males was 1 in 1,212 or 0.0823 per cent. On the other hand the frequency of non-disjunction females was 7 in 857 or 0.82% which is a hundred times greater. The non-disjunction must occur coincidentally with the segregation of the $2.Y$ chromosome in the same cell in order to be detected.

DISCUSSION

The variegation exhibited by the 10C-2 mutual translocation between 2R and the Y chromosome is the result of a break very near the *brown* locus and placing the heterochromation of the Y chromosome adjacent to the *brown* locus. This locus (2-104.5) was first shown by Glass (1933) to be sensitive to chromosomal rearrangements. Slatis (1955a) stated that the *brown* locus is most likely in the region between 59D8 and El-2 on Bridges map, but Demerec (1941) gave information indicating that the locus might be anywhere within region 59. The break in 10C-2 occurred at 59C6.

SUMMARY AND CONCLUSIONS

1. The 10C-2 stock contains a mutual translocation between the right arm of the second chromosome and the long arm of the Y chromosome.
2. The variegation or mottling is less obvious in females than in males.
3. Any male containing a $Y.2$ chromosome without a $2.Y$ chromosome is viable but sterile.
4. Males containing both $Y.2$ and $2.Y$ chromosomes are viable and fertile.
5. A fly (male or female) is inviable when it contains a $2.Y$ chromosome without a $Y.2$ chromosome.
6. Only those flies containing both $2.Y$ and $Y.2$ chromosomes have variegated eyes.
7. Appropriate crosses can produce flies which are homozygous for both $Y.2$ and $2.Y$ chromosomes. Such individuals have almost solid brown eyes, in which the variegation is barely detectable.
8. Non-disjunction between the X and $Y.2$ chromosomes occurs occasionally, producing a variegated female.

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Telomeres and Terminal Chiasmata—A Reinterpretation

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The phenomenon of chiasma terminalization (movement of the chiasmata, or some of them, towards the ends of the chromosomes) is a very general, but probably not universal feature of the late prophase of meiosis. First clearly recognized as a distinct process by Darlington (1929), it has recently been discussed by Swanson (1957, pp. 214–218). Darlington (1932) defined the process as “expansion of the association of the two pairs of chromatids on one side of a chiasma at the expense of that on the other side.” When a chiasma terminalizes right to the end of a bivalent it becomes a “terminal chiasma,” i.e., an association of two chromosome ends (each consisting of two chromatids) with a double connecting thread between them. In some species a chromomere or minute dark-staining enlargement is seen in the middle of each connecting thread (Fig. 1).

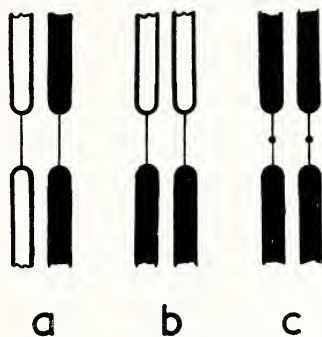


FIG. 1. Terminal chiasmata. Maternal and paternal chromatids indicated in *a* and *b*. On the interpretation put forward here, all terminal chiasmata are of type *a*; on Darlington's interpretation a configuration like *b* can arise through terminalization of a pair of compensating chiasmata to the same chromosome end. *c*, a terminal chiasma with chromomeres in the middle of the connecting threads (presumably each such chromomere consists of two halves, one belonging to each chromosome).

Usually, the connecting threads between the chromosome ends are broken at first anaphase. But in some Hemiptera that have pre-reductional separation at the terminal chiasma (White, 1954, fig. 97) the chromatids are associated in pairs by “half terminal chiasmata” all through the succeeding interphase and until the second anaphase.

The theory of terminal chiasmata which was put forward by Darlington was that a special “terminal affinity” existed between chromosome ends. This force of attraction was supposed to develop *after* the early diplotene stage, because if it were present at the beginning of diplotene, *all* chromosome ends would remain associated and every bivalent would show two terminal associations in addition

¹ Dedicated with esteem and admiration to Professor J. T. Patterson—and in memory of the years 1947–1953 spent by the author at the University of Texas.

to true interstitial chiasmata. This assumption of a special affinity between chromosome ends, which manifests itself only at a particular stage of meiosis and presumably only following chiasma formation, seems to be a very special and unlikely one. It was put forward before the difference between the properties of natural chromosome ends or telomeres and freshly broken ends had been recognized (Muller, 1932, 1938).

The essential argument on which the idea of terminal affinity is based rests on the "observation" that compensating chiasmata (i.e. reciprocal or complementary pairs—two strand doubles and four strand doubles in the terminology of the *Drosophila* workers) do not "cancel out" when they reach the same chromosome end—i.e., that when a compensating pair of chiasmata terminalize to the same end the result is a terminal association ("terminal chiasma") and not a mere falling apart of the ends.

But is this really so? No critical evidence ever seems to have been presented on this point. Most cytologists seem to have accepted Darlington's interpretation, probably because they have assumed that (1) compensating pairs of chiasmata are common, (2) terminalization is common, (3) falling apart of chromosome ends at diplotene to first metaphase is very rare or non existent.

In figure 2 we show an example of a cell which we interpret as containing a pair of chromosomes which were held together at diplotene by a pair of compensating chiasmata but have cancelled out by first metaphase. That such cells are not common is understandable; if they were, a significant amount of non-disjunction would undoubtedly result in most types of meiosis. If they occur at

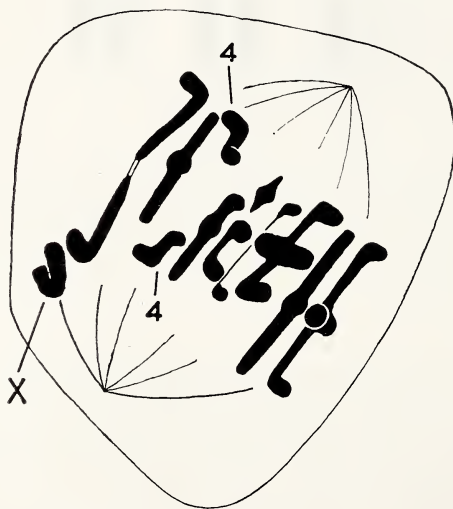


FIG. 2. A first meiotic metaphase (in side view) in an individual of the grasshopper *Trimeroptis gracilis*. This particular individual was heterozygous for the Rochester and Standard sequences of chromosome 7. Chromosome 4 consists of two univalents situated opposite one another, as if they had been paired at an earlier stage. It is suggested that they were associated by a pair of reciprocal chiasmata in the same arm which "cancelled out" at premetaphase. But on account of the hooked shape of the distal ends of the separating chromosomes it cannot be regarded as certain that the two chiasmata terminalized right to the end of the bivalent before cancelling out.

all, however, they are evidence against Darlington's theory of the terminal chiasma, at least in its original form.

Callan (1949) in a popular article put forward very briefly a suggestion which amounts to a radical alternative to Darlington's theory of terminal chiasmata. Because of the way in which it was published, this suggestion seems not to have received the attention it deserves. Although we do not think it can be accepted precisely in its original form, we believe it points the way to a correct interpretation.

Briefly, Callan's suggestion is that the chromosome tips are physically undivided at the stage when terminal chiasmata are seen. If this were so, a terminal chiasma would indicate "equational" separation of chromosome ends. It would result whenever a single chiasma became terminalized—or when a pair of diagonal chiasmata terminalized to the same chromosome end. On the other hand, if two compensating chiasmata were to terminalize to the same end there would be no terminal association formed and the ends would simply fall asunder "reductionally" (which is what we believe has happened in the cell illustrated in figure 2).

The idea of the chromosome end or telomere being simply undivided at a time long after replication of the rest of the chromonema has been completed is possibly a somewhat awkward one from the biochemical standpoint. But there seems no biochemical objection to some kind of sister strand union between chromatid ends that have already replicated. But the chemical bonding involved must be more labile than in true sister strand union; since it is broken at the end of first metaphase, when the chromosome ends are torn asunder on the elongating spindle. There is no absolutely convincing evidence whether the breaking apart of the associated chromosome ends is due simply to tension imposed by stretching of the spindle, or whether some kind of enzymatic action is involved which breaks the special "telomeric bonding." But in certain organisms at any rate (Schrader, 1944; Hughes Schrader, 1947; Staiger, 1954) the telomeric bonding which we postulate seems able to withstand a very violent stretching at premetaphase and is then broken at the end of metaphase when stretching is apparently much less violent. We may therefore suspect that some specific chemical mechanism (a "telomere-ase") is responsible for breaking the telomeric bonds at the beginning of first anaphase.

Another reason for believing that it is not mechanical tension which breaks the telomeric bonding is that metacentric chromosomes appear X-shaped at interkinesis even when there has been a chiasma in one arm only. The fact that the telomeric connections at meiosis are able to withstand the premetaphase tension suggests that relatively strong chemical bonding is involved; possibly disulfide bridges, since the structure would almost certainly have to be a symmetrical one. If the chromosome consists of many short segments bound together by calcium bridges (Mazia, 1954), then the telomeric connections are probably *not* due to calcium.

If telomeres consist essentially of a special kind of sister strand union at meiosis (or of chemical groupings capable of taking part in such unions), it is logical to suppose that the telomeres of somatic chromosomes are essentially similar. The existence of telomeres was postulated in the first place because natural ends of

chromosomes show no tendency to fuse with one another, or to undergo true sister-strand union—properties exhibited by artificial ends produced by chromosome breakage. That telomeres should be unable to fuse in these ways is readily understandable if they consist, essentially, of a different kind of sister strand union, of a more labile type. At what stages of the mitotic cycle the telomeric connections would be between chromatids and at what stages they would be between sub-chromatids is not clear. But presumably, sub-chromatid connections replace chromatid unions at a particular stage, probably during interphase.

If the above hypothesis is essentially true, the chromosome ends produced by radiation breakage and those which result from mechanical breakage (stretching on the spindle) lack telomeric properties because they are incapable of forming the special labile type of sister-strand union. Instead, they form unions of a more permanent kind which are not broken at each mitotic and meiotic cycle. "Healing" of broken ends (such as occurs in maize sporophytes, according to McClintock, 1942, and in the somatic chromosomes of *Ascaris* during the cleavage divisions) would represent a change at the broken surface, probably either a loss or a gain of some material, which leaves the exposed end able to take part in a telomeric type of sister strand union.

Various cytologists have claimed to have seen telomeres as specially staining structures, at particular stages of mitosis or meiosis. But obviously there can be no critical evidence that what they saw were the telomeres themselves rather than heterochromomeres or other structures immediately proximal to them. The same argument applies to the chromomeres sometimes seen in the middle of the threads connecting chromosome ends united in a terminal chiasma (fig. 1c). All that we are justified in concluding in the latter case is that the region immediately proximal to the terminal chromomere possesses considerable elasticity, so that it can be greatly extended—not that the terminal chromomere is itself the telomere. The latter may be a single nucleotide or nucleotide pair, a single amino acid or even an exposed sulfhydryl group at the tip of the chromosome. Terminal adhesions between chromosome ends in polytene nuclei or at meiosis, such as have been reported for various organisms, are more likely to be due to the terminal chromomere, or to several chromomeres at the end of the chromosome rather than to the telomere in the strict sense we have been considering here.

It is an observed fact that terminalization is usually much more extreme in organisms having a maximum of one chiasma per chromosome arm. On the supposition that compensating chiasmata do not "cancel out" in terminalization, this fact was unexplained. But if they can do so, it is understandable that terminalization should be absent, poorly developed or confined to the distal chiasma (or, alternatively, to the proximal ones) in species with more than one chiasma per chromosome arm. If it were otherwise "accidents" such as that shown in figure 2 would occur and meiotic nondisjunction would be too frequent. Thus we believe that regularity of disjunction is best served by extreme terminalization only where there is a maximum of one chiasma per arm and that selection has operated to produce these regularities of the meiotic mechanism.

The ideas expressed here are put forward in a tentative manner only, as an attempt to re-open discussion on some fundamental problems of chromosome behavior. With the development of techniques for isolating the whole mitotic

apparatus it is not too much to hope that some of these problems may soon yield to an experimental approach.

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Dominant Lethal Mutation in Irradiated Oocytes¹

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Patterson, Brewster and Winchester (1932) in experiments dealing with dominant lethal effects, chromosome losses, etc., demonstrated some of the possibilities for using *Drosophila* females in the study of radiation effects on genetic material. It is advantageous to be able to recover eggs known to have been in meiotic prophase when treated, and, since the completion of meiosis depends on sperm entrance, it is possible by holding females virgin to delay completion of this process by several days. Patterson and coworkers were able to show a considerable increase in radiation sensitivity with age. It is unfortunate that the significance of this frequently has been overlooked.

Fuller utilization of *Drosophila* females as experimental material awaited the development of a simple procedure for screening for events depending on chromosome breakage and rejoining. The successful interpretation of the nature of radiation-induced detachment of attached-X's made independently by Parker (1953, 1954) and by Herskowitz and Muller (1953) and Herskowitz (1954) provided the needed technique. The morphological description of oogenesis by King, Robinson and Smith (1956) has been found useful in work on the effects of aging on sensitivity. There has been good agreement between their descriptions of the distribution of stages and experimental findings in this laboratory.

It is possible to distinguish at least four different levels of response in oocytes. The highest sensitivity is found in the oldest cells in females aged three or more days after eclosion. The oldest cells in newly emerged females show lower frequencies of rearrangement and of both dominant and recessive lethals. The next preceding stage will show about the same level of rearrangement (ie. detachment of attached X) at lower doses but will be destroyed by higher dosages (eg 4000 r). Younger than these are stages in which crossing over may be induced, but with no appreciable frequency of induced detachment of attached X's (Parker, in discussion of Whittinghill, 1955). The most useful stages are the ones designated as stages 7 and 14 by King, et al. The oldest oocytes in newly emerged females are in stage 7, while virgin females aged two or more days have one stage 14 oocyte in each ovariole. Thus, by limiting the number of eggs collected from any one female, a fairly homogenous sample of cells may be obtained.

The differences in response of stages 7 and 14 to irradiation are both qualitative and quantitative. Stage 14 shows a higher incidence of all types of genetic damage which have been looked for, with break-rejoining delayed until fertilization, while breaks induced in stage 7 rejoin in about 10-15 minutes (Parker 1955; Parker and Hammond, 1958; King, Darrow and Kaye, 1956). In stage 7 most interchromosomal exchanges involved homologues or chromosomes which

¹ The experimental work reported here was carried out at the University of Mississippi, and was supported by Contract No. AT(40-1)-2163 with the U. S. Atomic Energy Commission.

presumably are partially homologous, as X and fourth chromosomes (Sandler and Novitski, 1956), while stage 14 yields relatively more interchanges between nonhomologous chromosomes (eg., X and second chromosomes) and more gross deficiencies (Parker and McCrone, 1958). Similarly, because of differences in shapes of the survival curves, there is reason to believe that the mechanisms of origin of dominant lethals may differ in these two stages (Parker, 1955; King, 1957).

A considerable literature has developed on hypotheses attempting to relate the origin both of dominant and of recessive lethals to chromosome breakage and rejoining. The resolution of these problems has been delayed in part by difficulties in interpretation of events taking place in mature *Drosophila* sperm. Perhaps the different conditions found in oocytes will permit the development of a fruitful approach to the problems of lethal mutation. This report represents a first attempt on the part of this writer to develop such an approach.

TECHNIQUES OF EGG COUNTING

In determining the frequencies of dominant lethals, eggs were collected from individual treated or untreated Oregon-R females over a period of approximately 24 hours. Where aged females were treated, these were mated immediately with two Oregon-R males each. Females treated 0–12 hours after emergence were stored at room temperature (74°) for two days, then mated with two Oregon-R males each. In earlier experiments (Table 1) matings were made in 25 x 95 mm vials containing the ordinary culture medium of the laboratory to which powdered charcoal had been added to give a light gray color to facilitate egg counting. In all later experiments, because of difficulties in making counts in these vials, etherized flies were placed in empty vials which were bound together in groups of seven and inverted on petri dishes containing the charcoal medium. Following a suggestion of Dr. C. W. Edington, mycostatin was used to inhibit yeast growth. A solution of 250,000 units of the antibiotic in 200 ml of 50% ethyl alcohol was sprayed lightly on the surface after eggs had been laid. About 26–28 hours were allowed for egg hatching, after which egg counts were made, tabulating totals of hatched and of unhatched eggs. Where necessary, plates could be stored at about 5° until counts could be made. At this temperature, larvae are killed and counts can be delayed without affecting the accuracy of the data. Mycostatin was not found to affect egg hatchability, as Oregon-R gave $95.4 \pm 1.2\%$ hatch with mycostatin and $95.2 \pm 1.1\%$ without.

The number of eggs any individual female could lay for the count to be tabulated was 10–30 inclusive. Any group of eggs in which none hatched was considered the product of an uninseminated female. Any error introduced by this assumption, at the dosage used, would be smaller than the one introduced by considering this the result of complete killing. Where more than 30 eggs were laid, some may have been in earlier stages at the time of treatment. As there were few cases of exclusion of counts, about equally frequent at each dosage used, these exclusions do not change the percentage hatch very appreciably.

DOSE RESPONSE IN DOMINANT LETHAL PRODUCTION

The first series of egg counts involved stage 7 material which had been irradiated with 250 KVP X-rays, with 3 mm Al-filtration, at a dose rate of about 300 r/minute. Through an error in calibration of the dosimeter, there is probably an error in total doses given, but the error factor would be common to all points. The remaining treatments, both stage 7 and 14, were given with a Snook machine operated at 60 KVP (1 mm Al-filter) at a dose rate of 100 r/minute. All treatments were based on readings taken with a Victoreen r-meter which had been calibrated against a known Cobalt-60 source at Oak Ridge National Laboratory. The results are given in Table 1. Figure 1 shows the shapes of the survival curves, and includes points plotted from the data of Sonnenblick (1940) for stage 14. There is a marked difference in sensitivity of the two stages, as well as in shapes of the two survival curves. These have been interpreted as "two-hit" and "one-hit" curves, respectively for stage 7 and stage 14. Sonnenblick's points show agreement with the theoretical curve at lower doses. Above about 1000 r, his points lie above the line, giving a curve suggesting a mixture of cells with

TABLE 1
Dominant lethal dose response

Stage Treated	Dose in r-units	Number Eggs Laid	Number Eggs Hatched	Percentage Hatch $\pm$ S.E.	Corrected Percentage Hatch
7	control	467	456	97.6 $\pm$ 0.7	100.0
7	900	466	444	95.3 $\pm$ 1.0	98.7
7	1,800	317	271	85.5 $\pm$ 2.0	87.6
7	3,600	318	184	57.9 $\pm$ 2.8	59.3
7	control	392	358	93.9 $\pm$ 1.2	100.0
7	1,500	549	459	83.6 $\pm$ 2.0	89.0
7	3,000	424	269	63.3 $\pm$ 2.3	67.4
7	6,000	197	64	32.5 $\pm$ 3.3	34.6
7	control	447	415	92.8 $\pm$ 1.2	100.0
7	700	930	848	91.2 $\pm$ 0.9	98.2
7	1,400	517	428	82.8 $\pm$ 1.7	89.2
7	2,800	765	487	63.7 $\pm$ 1.7	68.6
7	4,900	543	173	31.9 $\pm$ 2.0	34.3
14	control	395	374	94.7 $\pm$ 1.1	100.0
14	250	522	357	68.4 $\pm$ 2.0	72.2
14	500	279	97	34.8 $\pm$ 2.9	36.7
14	control	1,054	952	89.5 $\pm$ 0.9	100.0
14	50	574	503	87.6 $\pm$ 1.4	97.9
14	100	568	430	75.7 $\pm$ 1.8	84.6
14	150	383	265	69.2 $\pm$ 2.4	77.3
14	200	645	405	62.8 $\pm$ 1.9	70.2
14	250	252	146	57.9 $\pm$ 3.1	64.8
14	300	400	208	52.0 $\pm$ 2.5	58.1
14	500	233	83	35.6 $\pm$ 3.1	39.8
14	600	321	80	24.9 $\pm$ 2.4	27.8

FIGURE 1

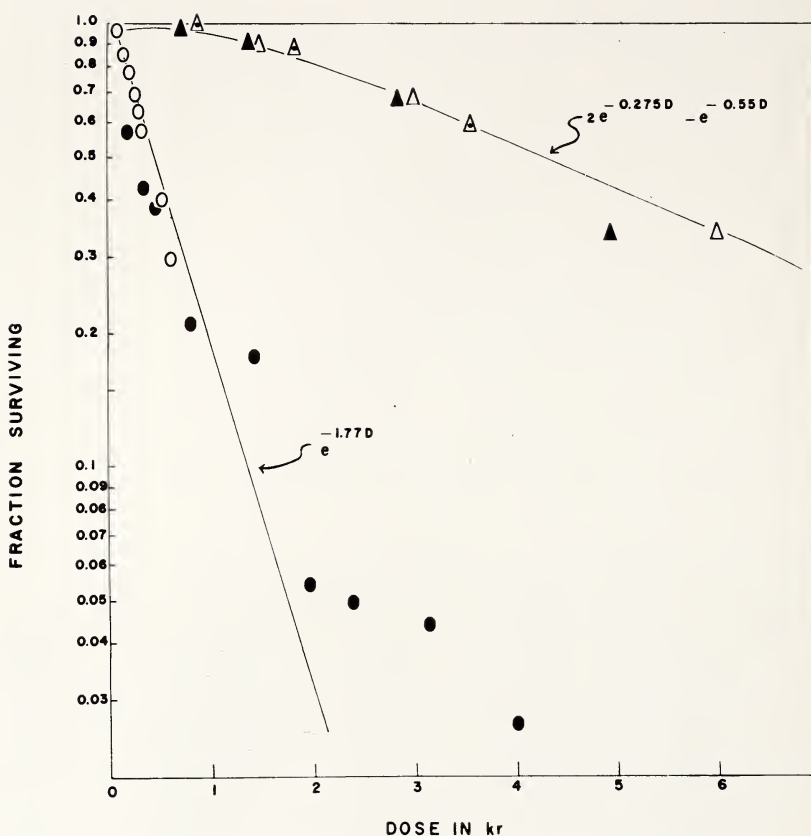


FIG. 1. Survival curves for irradiated oocytes. Triangles represent three replications of the experiment irradiating stage 7 material. Circles represent irradiated stage 14 material, the filled circles being taken from the data of Sonnenblick (1940).

two markedly different sensitivities. His egg collections may have included a few which had been pre-stage 14 when treated. King, Darrow and Kaye (1956) show the same types of curves, again with a marked concavity in the stage 14 curve.

EFFECT OF VARYING THE INTENSITY OF IRRADIATION ON SURVIVAL

Knowledge of the time required for chromosome break rejoining in the two treated stages of oogenesis would allow some predictions to be made of effects of dose-fractionation on the frequency of dominant lethals which might depend on the rejoining of broken ends of chromosomes. Parker and Hammond (1958) concluded that breaks in stage 7 rejoin in about 10–15 minutes; those in stage 14 rejoin about the time of fertilization. Thus, the conditions in stage 7 would lead one to expect an intensity effect if the “two-hit” dominant lethals depended on the interaction of two independently induced breaks. On the other hand, failure

of early rejoining of breaks in addition to the "one-hit" nature of the response in stage 14 would lead to the prediction of no intensity effect.

Irradiations in this series were made with a G. E. Maximar-100 machine operated at 100 KVP with a 1 mm Al-filter, giving a HVL of 1.3 mm Al. Dosages were monitored as in the previous irradiations. The dose rate was approximately 300 r/minute. The dosage used in stage 7 was 3000 r, while that in stage 14 was 300 r. These were chosen as each will give around 60 percent survival. Treatments were either given in one continuous dose or were divided into two equal fractions with a measured time interval between treatments. Table 2 gives the

TABLE 2

Effects of dose fractionation on dominant lethality: Dosages divided in two equal fractions separated by variable time interval

Stage Treated	Total Dose	Interval Between Fractions	Number of Eggs Laid	Number of Eggs Hatched	Percentage Hatch $\pm$ S.E.	Percentile Increase
7	3,000	0	1,091	599	54.9 $\pm$ 1.1	
7	3,000	45 min.	1,219	753	61.8 $\pm$ 1.4	6.9 $\pm$ 1.8
7	0	..	172	161	93.6 $\pm$ 1.9	
7	3,000	0	350	202	57.7 $\pm$ 2.6	
7	3,000	15 min.	305	200	65.4 $\pm$ 2.7	7.7 $\pm$ 3.8
7	3,000	30 min.	277	156	68.7 $\pm$ 3.1	11.0 $\pm$ 4.1
7	3,000	60 min.	309	222	71.8 $\pm$ 2.6	14.1 $\pm$ 3.7
7	0	..	721	693	96.1 $\pm$ 0.7	
7	3,000	0	2,454	1,460	59.5 $\pm$ 1.0	
7	3,000	30 min.	2,848	1,905	66.9 $\pm$ 0.9	7.4 $\pm$ 1.3
14	300	0	961	524	54.5 $\pm$ 1.7	
14	300	15 min.	828	456	55.1 $\pm$ 1.7	0.6 $\pm$ 2.4
14	300	30 min.	1,045	558	53.4 $\pm$ 1.5	-1.1 $\pm$ 2.3
14	300	0	2,447	1,320	53.9 $\pm$ 1.0	
14	300	4 hours	619	300	48.5 $\pm$ 2.0	-5.4 $\pm$ 2.2

results of these treatments. There seem to be effects of the type expected, with significant increases in percentage of hatch in the stage 7 series where the interval between fractions was long enough (10-15 minutes) to allow restitution and rejoining to occur in the first breaks produced before the second group were induced. As expected, fractionation did not increase survival in stage 14. The control values (continuous treatment) are somewhat lower in the stage 7 material than those given in Table 1. Perhaps this was due to the higher intensity used (300 r/minute vs. 100 r/minute).

CENTRIFUGATION

Sax (1943), and Wolff and von Borstel (1954) have shown that aberration frequency in *Tradescantia* and in *Vicia* may be increased by post irradiation centrifugation which might move broken ends of chromosomes apart making restitution less likely, while centrifuging before irradiation lowered aberration

frequency, presumably by a packing effect which lowered mobility of the chromosome fragments produced by subsequent irradiation.

Drosophila females can withstand centrifugal forces of the order of 1200 g without any observable effect on fecundity or hatchability of non-irradiated eggs. Small numbers of females were placed in a paper capsule closed with a soft cotton plug. The capsule was then placed in the centrifuge tube with the plug down. The centrifuge used had a radius of six inches, and was operated at approximately 2600 rpm for 5 or for 20 minutes giving a RCF of about 1150 g. Flies were centrifuged either immediately before or after irradiation, otherwise the conditions of irradiation were similar to those in the fractionation series: Stage 7 was given 3000 r; stage 14 received 300 r. The results are found in Table 3.

TABLE 3
Effects of centrifuging (1150 g) on the induction of dominant lethals.
(B, before and A, after irradiation)

Stage	Dose	Centrifuging	Number Eggs Laid	Number Eggs Hatched	Percentage Hatch $\pm$ S.E.	Percentile Decrease
7	0	20 min.	1,177	1,101	93.5 $\pm$ 0.7	
7	3,000	none	3,153	1,701	53.9 $\pm$ 0.9	
7	3,000	A-20 min.	2,329	931	40.0 $\pm$ 1.0	13.9 $\pm$ 1.3
7	3,000	none	1,188	733	61.7 $\pm$ 1.4	
7	3,000	A-20 min.	818	381	46.6 $\pm$ 1.7	15.1 $\pm$ 2.2
7	3,000	B-20 min.	800	471	58.9 $\pm$ 1.7	2.8 $\pm$ 2.2
7	3,000	none	827	423	51.2 $\pm$ 1.7	
7	3,000	A-5 min.	1,043	529	50.7 $\pm$ 1.5	0.5 $\pm$ 2.3
14	300	none	1,648	770	53.3 $\pm$ 1.3	
14	300	A-20 min.	1,661	827	49.8 $\pm$ 1.2	3.5 $\pm$ 1.8

No effect of pre-irradiation centrifuging can be seen, while post-irradiation centrifuging resulted in a lowered hatchability of stage 7 only. Since few inter-chromosomal exchanges involving non-homologous chromosomes are produced in this stage, little "packing effect" would be expected, and the absence of a pre-irradiation centrifugation effect is not too surprising. Breaks in stage 14, on the other hand, remain open for a long time after being centrifuged, and the small amount of movement resulting from centrifugation might have little effect on the distance between broken ends at a time when rejoining becomes possible. The results are, therefore, compatible with the idea that chromosome breakage is a factor in the production of a significant fraction of dominant lethals.

HYPOTHESES OF DOMINANT LETHALS

If dominant lethals arise as a result of chromosome breakage, what events must follow for the lethal change to be effected? The low frequency of inter-chromosomal aberrations induced in females (eg. Glass 1955) suggests that mechanisms involving aneupentric rearrangements between nonhomologous

chromosomes play at best only a minor role in these cases. Particularly must this be the case with the "one-hit" dominant lethals in stage 14, the stage where a somewhat higher frequency of translocation between nonhomologous chromosomes might be expected (Parker and Hammond, 1958). Precisely the same argument can be applied to large deficiencies.

The suggestion was made by A. R. Whiting (1945) for *Habrobracon*, that dominant lethals may arise through formation of bridges in anaphase II of Meiosis. This interpretation was later adopted as a working hypothesis by Parker (1955) and in a modified form by King (1957).

The strong inference that rearrangement depends in part on the proximity of breaks, leading to the low incidence of translocation in females, would permit the occurrence of rearrangements involving breakage and rejoining in chromatids, either sisters or homologues in early prophase; in late prophase perhaps only sisters or chromatids effectively behaving as sisters, i.e. homologues lying together distal to a crossing-over point but proximal to the resultant terminalizing chiasma.

An exchange giving rise to a dicentric chromosome and an acentric fragment might yield a dominant lethal especially if the resulting bridge were in anaphase II and involved the prospective egg nucleus. Anaphase I bridges might be eliminated in the same manner as when formed by crossing over in inversion heterozygotes (Sturtevant and Beadle, 1936), although anaphase I bridges formed in autosomes might not be eliminated since apparently only acrocentric chromosomes give this result (Novitski, 1952).

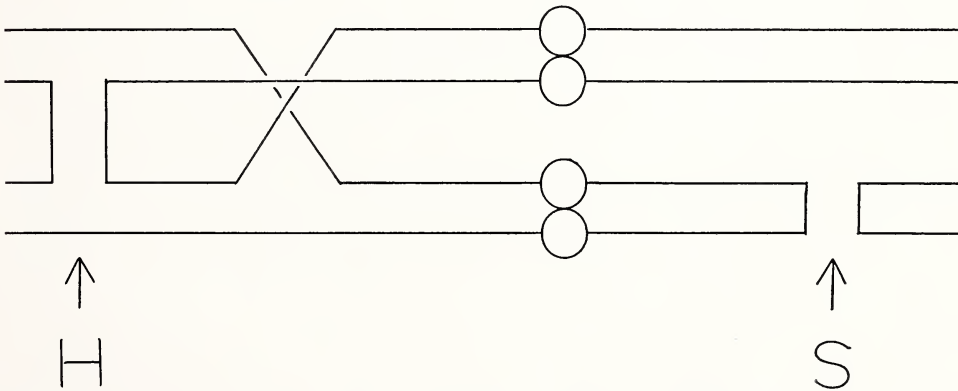


FIG. 2. Two possible modes of origin of Anaphase II bridges. S, sister union; H, aneucentric rejoining of homologues distal to a chiasma.

THE PRODUCTION OF COMPOUND X'S BY IRRADIATION

The description by Novitski (1954) and Sandler (1954) of the origin of reversed acrocentric compound-X chromosomes suggested a technique of approach. Reversed acrocentrics arise spontaneously with a low frequency in females heterozygous for a long inversion (scute⁸) if the normal-sequence X chromosome has the long arm of the Y attached. In the absence of an attached arm, this type of recombination does not take place. A great many of Sandler's

cases showed homozygosis for markers near the point of union of the two X's, whereas the mother had been heterozygous for those loci. This showed that two meiotic events had been necessary for the origin of these chromosomes: sister union in the proximal heterochromatin of one, with an appropriate crossover distal to that point.

Since the hypothesis of dominant lethals involves the formation of sister unions following breakage of two sister chromatids, the irradiation of inversion heterozygotes with no attached Y arms suggested itself as a means of testing for these radiation-induced sister unions.

Females of the composition $\gamma\ car\ bb/ sc^s\ w^a\ cv\ v\ f$ were irradiated and mass mated to "Muller 5" ($sc^{s1}\ B\ In\ S\ w^a\ sc^s$) males, and allowed to lay eggs for 24 hours. These females were irradiated either within 12 hours after emergence, or 5 days later, giving results on stage 7 and stage 14 oocytes respectively. All non-Bar female offspring were tested for the presence of a compound X by remating to Muller 5 males—these females were recorded as to phenotype, and for loci which became homozygous in subsequent generations. Various dosages were used in the treatments in the hope of distinguishing "one-hit" and "two-hit" effects.

Two series of irradiations were made on Stage 7 material, and one on stage 14. The results of these irradiations are given in Table 4.

It would be difficult to conclude from these data whether or not there is a two hit effect in stage 7. In both series, at lower doses the increases in frequency with dose seems to exceed the linear expectation, however, at higher doses this is not the case. From only two points, it would be impossible to interpret the

TABLE 4
Induction of compound X's by irradiation of inversion heterozygotes.
(*In* (1) $sc^s\ f\ v\ cv\ w^a/\gamma\ car\ bb \times$ Muller-5 ($sc^{s1}\ B\ In\ S\ w^a\ sc^s$)

Stage Treated	Dose	Regular Classes		+	Compound X			Non-disjunctional Classes		Percentage Compound X's
		Bar♀♀	Non-Bar♂♂		y	yf	y car	Non-Bar♀♀	Bar♂♂	
7	0	508	421	.	.	.	.	.	.	
7	1,000	720	579	.	.	.	.	1	.	
7	1,500	1,438	1,281	.	1	.	1	5	3	0.074
7	2,000	438	346	.	1	.	1	.	.	0.25
7	3,000	1,679	1,061	1	7	2	.	13	4	0.36
7	4,000	256	151	.	2	.	.	.	.	0.49
7	6,000	120	76	.	.	1	1	1	1	1.0
7	750	6,990	4,610	.	1	.	.	20	9	0.008
7	1,500	7,072	4,655	.	2	3	3	6	15	0.07
7	2,050	5,702	3,883	.	4	1	.	42	7	0.05
7	3,000	4,366	2,879	.	4	3	1	38	31	0.11
Totals for stage 7				1	26	10	12			
14	250	7,999	6,096	.	3	7	3	2	151	0.09
14	500	2,615	1,787	.	.	3	2	4	104	0.11
Totals for stage 14				.	3	10	5			

response of stage 14. However, as pointed out in the preliminary report (Parker, 1957) the data are not inconsistent with the expectations of two-hit and one-hit for stages 7 and 14 respectively.

It is impossible at present to make any valid estimate of the frequency of dominant lethals arising as a result of similar breakages and rejoinings. Meiotic drive (Sandler and Novitski, 1957) must limit the recovery of compound X's. Variations in the coefficient of non-randomness found in experiments of Novitski (1951), Novitski and Sandler (1956), from a low of about .67 to a high of at least .92 (with the possibility of much higher values not yet detected) would not allow a specific estimate of meiotic drive in the present case.

It would be necessary, further, to make corrections for crossing over distal to the unions to get a measurement of their frequency in the case of compound X's, while crossing over proximal to these would affect their fates to dominant lethality.

The events, to give rise to recoverable compound X's, must occur in heterochromatin and the resulting females must be fertile. These events seem to occur with different frequencies in the two X chromosomes used. There is no basis for estimating the frequency of their occurrence in euchromatic parts of chromosomes.

However, in spite of these difficulties, some conclusions can be drawn. The fate of these unions should not be affected by the time of their production, as in neither case is meiosis carried beyond metaphase I before these events have occurred. There should, therefore, be similar differences in sensitivity of stage 7 and of stage 14 oocytes, whether measured by dominant lethals or by production of compound X's. At the dosages used, for dominant lethals, doses giving similar survival differ by about a factor of 10, while for compound X production, there is about a three or four fold difference.

The compound X's heterozygous for all markers occurred largely in the stage 7 irradiations, there being 27 out of 49 in this stage as opposed to 3 out of 18 in stage 14. This might be expected if stage 14 represents late diplotene or diakinesis, with only sister chromatids being in contact in the proximal heterochromatin, and if stage 7 represents a much earlier prophase where wide separation of homologues has not yet occurred.

One apparent anomaly is the high frequency of *carnation* homozygotes as compared to *forked*. The presumed origin of these is given in Figure 3. Recovery of the *forked* class (S_2) should be favored since the compound X will be separating at anaphase II from an anaphase I bridge. On the other hand, the *carnation* class would be separating at anaphase II from *scute* 8, and its recovery would be affected adversely by meiotic drive.

DISCUSSION

Lea (1946) summarized the evidence available to him, concluding that much of the dominant lethal damage to irradiated sperm resulted from chromosome breakage. A. R. Whiting (1949) found that heavily irradiated eggs when fertilized by untreated sperm would give rise to patroclinous males, showing that death at ordinary dosages resulted from nuclear rather than from cytoplasmic

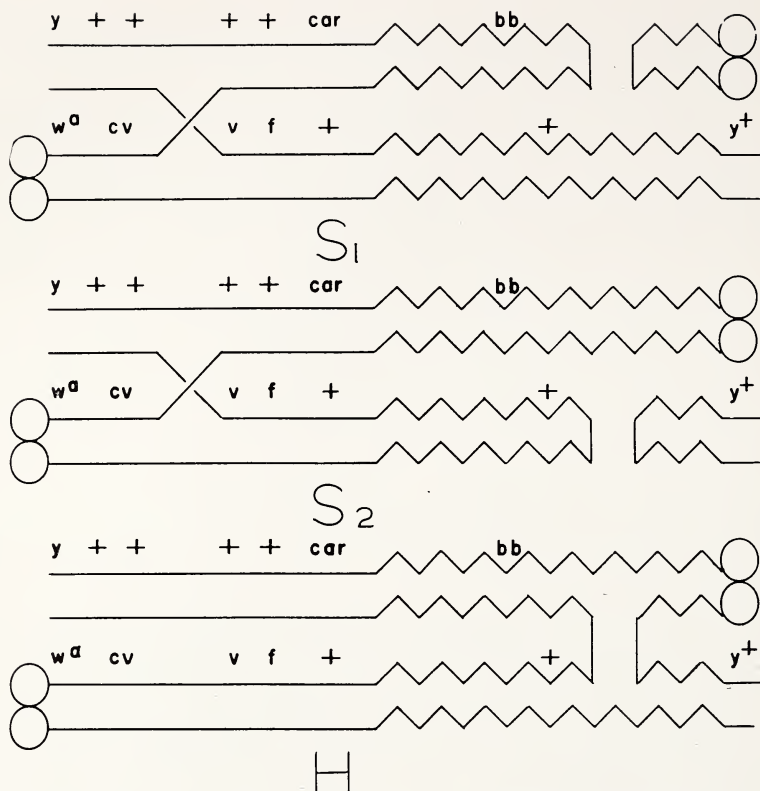


FIG. 3. Possible modes of origin of compound X's by irradiation of inversion heterozygotes. S_1 , sister union in the normal sequence; S_2 , sister union in the inverted sequence; H , eucentric rejoining of homologues.

damage. Also in *Habrobracon*, von Borstel and Moser (1956) were able to show that for lethal effect with ultra-violet the nucleus was more sensitive than the cytoplasm. Schwartz and Bay (1956) show that radiation doses high enough to prevent mitosis will allow cells to survive and for a while to carry on otherwise relatively normal metabolic activities, implying that death results from chromosome damage which is expressed when the cell divides.

On the other hand, von Borstel and Pardue (1956) and von Borstel (1958) have evidence that most radiation-induced dominant lethal effects occur earlier in development than does death from aneuploidy in the progeny of non-irradiated translocation heterozygotes and triploids. They suggest that dominant lethality may be associated with interference with DNA synthesis. However, their aneuploids differ from the kind postulated by classical dominant lethal theory: no anaphase cleavage bridges were involved. Until this difficulty can be overcome their argument must remain unanswered. It may be possible by studying the time of death of progeny of various kinds of compound X's where it is known that crossing over will produce anaphase II bridges with various frequencies (Novitski, 1954, 1955) to see where in ontogeny death will occur.

Herskowitz and Schalet (1956, 1957) have presented evidence which they

believe will account for at least $1/9$ of the dominant lethal damage induced at 2000r as being the result of "half-translocation" and of non-disjunction and chromosome losses. Their estimate may be a good one in that it is not inconsistent with the figures given by von Borstel and Pardue (1956), where about 80 percent of dominant lethals induced in *Habrobracon* oocytes die early as do about 90 percent of those induced in *Drosophila* sperm, presumably leaving about 10–20 percent for later death which could be due to simple aneuploidy. However, dominant lethals of this sort cannot account for the majority of deaths actually induced. It is definite that the response of stage 14 is "one hit," hence we may rule out "two hit" events as accounting for any appreciable number of dominant lethals in this stage. (Table 1; also King, Darrow and Kaye, 1956.) On the other hand, stage 7 gives "two hit" dominant lethals, as shown by the dose response curve and by the intensity effect (Tables 1 and 2; King, Darrow and Kaye, *ibid*). However, the low frequency of interchromosomal rearrangements induced in this stage (Parker and McCrone, 1958) would tend to rule out the significance of the contribution of "half translocations" to dominant lethality in this stage.

King (1957) has suggested a modified version of A. R. Whiting's (1945) hypothesis of induction of bridges in anaphase II of meiosis. He accounts for the origin of each bridge as a one hit event, and assumes that at least two bridges are necessary if induced by irradiation of stage 7, requiring the additional hypothesis that the egg nucleus is not oriented: either end nucleus may be able to function as the egg nucleus. He explains the "one hit" curve obtained with stage 14 as multiple breakage so that enough bridges are formed to insure some passing to opposite poles at anaphase I.

There are several reasons for believing this hypothesis incorrect. Novitski (1954) in work dealing with the fate of anaphase II bridges showed that the production of nullo-X eggs by tandem-ring compound-X females might be explained as the result of loss of the X due to the formation of double anaphase II bridges. He was able to show that symmetrical double bridges lag and are lost on the spindle while single and asymmetrical double bridges are lethal, presumably due to breakage and inclusion of a fragment in the egg nucleus. Since there is normal disjunction of compound X's at anaphase I, meiosis would always produce at least two bridge-free nuclei, and the phenomena of lethality and chromosome loss would not be expected unless one made the unlikely assumption that bridges arising by crossing over behave differently from radiation-induced bridges.

Evidence for the determination of the egg nucleus has been suggested by von Borstel (1957). Binucleate eggs occur occasionally in *Habrobracon*, while the corresponding binucleate oocyte has not been found. In stocks producing a high frequency of binucleate eggs (and in no others) meiosis frequently is not blocked at metaphase I, occurring in the interior of the egg rather than at the surface, allowing the possibility of survival of more than one nucleus. von Borstel postulates an agent, present in the cortical cytoplasm, which is responsible for disintegration of the polar nuclei, the egg nucleus escaping destruction by being pushed farther toward the interior by each of the meiotic divisions.

Were King's hypothesis of a non-determined nucleus correct, it would be difficult to explain the very low frequency of binucleate eggs in non-irradiated

Drosophila. Some binucleate eggs would give rise to gynandromorphs and/or autosomal mosaics. Mosaics arising in this manner are much rarer than those involving chromosome loss. While the recognition of these may depend on the two egg nuclei being non-sister (i.e., product of the same first but a different second meiotic division), a higher frequency of involvement of sister nuclei would be of little consequence to the hypothesis of dominant lethals, as these would be the ones involved in single anaphase II bridges.

Still another basis for at least partial rejection of King's hypothesis is the intensity effect reported here and by Abrahamson and Herskowitz (1955, 1957). If, as in King's hypothesis, all bridges are produced by "one hit" events, there would be no interaction between the two struck targets, hence one would predict a "two hit" curve but no intensity effect.

Sonnenblick (1940) dealt with egg samples that were largely in stage 14 at the time of treatment, and there is very good agreement between his data and those reported here. Lea (1946) has used his description as a basis for the hypothesis that no rejoining of breaks occurred in oocytes, giving rise to cleavage bridges following sister union occurring after the completion of meiosis. The evidence supporting this hypothesis was the failure to obtain translocations by irradiation of females (e.g. Glass, 1955). However, it was found that breaks induced in stage 14 could rejoin (Parker and Hammond, 1958; Parker and McCrone, 1958), and Parker (1955) was inclined to reject the Lea interpretation because of the incorrectness of this assumption. There are reasons for modifying this position. Since rejoining is delayed until the removal of the block on meiosis, and meiosis is then completed rapidly, perhaps some breaks miss an opportunity to rejoin at this time. The failure to find an increase in stage 14 in induction of compound X's comparable to that found in dominant lethals casts serious doubt on the hypothesis that in stage 14 most dominant lethals arise following sister fusion of isochromatid breaks. Perhaps most breaks are chromatid breaks, and many dominant lethals do arise in the manner suggested by Lea and by Sonnenblick. Parker and McCrone concluded that translocations induced in stage 14 involve chromatids, as these show evidence of meiotic drive as would be expected if the rearranged chromatid were separating from an intact one at anaphase II. If all or most of the breakage were isochromatid, one might expect either both sisters would be involved in each translocation, giving no basis for meiotic drive, or one sister might frequently fail to undergo restitution or rejoining and meiotic drive would operate in the opposite way to that actually found.

We should then expect "dominant lethals" to be a mixture of events, possibly not all involving breakage and/or loss of genetic material. Non-disjunction and losses of whole chromosomes may be radiation-induced and therefore contribute somewhat to dominant lethal effects. Meiotic drive will favor the aneuploid segregation of any translocation which is induced. It is impossible at this time to make any valid estimate of the frequencies of these events, but the evidence of von Borstel and Pardue (1956) must limit these to not more than 10-20 percent of all dominant lethals produced at ordinary dosages. Undoubtedly anaphase II bridge formation occurs, although again determination of its frequency is prevented by the phenomenon of meiotic drive as well as by lack of information on breakage and rejoining in euchromatin in oocytes. We may expect these single

anaphase II bridges to break (Novitski 1955) and their subsequent fate should be similar to that of broken chromatids which failed to reconstitute or rejoin before completion of meiosis. This final category of dominant lethals may constitute the great majority of the "one hit" deaths found in stage 14, and possibly make up a part of those produced in stage 7. This might account for the smaller than expected intensity effect found in this stage (Table 2). If failure to rejoin or reconstitute is a major cause of dominant lethality, then much of the difference in rate of production of dominant lethals in the stages worked with would be due to differential rejoining rather than breakage. Differences in the two stages may not be explainable on so simple a basis when rearrangement frequency is used as the measure of radiation sensitivity.

Because of the probable variety of events leading to dominant lethal mutation, it would be difficult at this time to develop a mathematical treatment of the phenomenon. An equation based on the dual target model of King (1957) may give a fairly good superficial fit to the data, but this may be spurious, due to intensity effects operating differently in treatments of different durations, relative amounts of "one-hit" and "two-hit" killing, and possibly a variety of other causes.

SUMMARY

Dominant lethals induced in stage 7 were found to follow a "two hit" curve, while those in stage 14 were "one hit." Evidence of participation of breakage and rejoining of chromosomes in the origin of dominant lethals is given by the finding that fractionation of the irradiation decreases while centrifuging after irradiation increases the incidence of dominant lethals in stage 7. Evidence that sister unions may occur following irradiation is given by the induction of compound X's by irradiation of inversion heterozygotes. This shows that anaphase II bridge formation might account for some dominant lethality. Dominant lethals must arise through a variety of mechanisms, and there must be differences in the relative frequencies of the various kinds in the two stages studied. It is not possible to estimate the frequencies of the various events in oocytes which would lead to dominant lethality, hence no mathematical treatment is attempted.

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Mammalian Chromosomes *in Vitro*. XI. Variability Among Progenies of a Single Cell¹

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Hsu and Klatt (1958) observed that in several cell strains of murine origin cells exhibited structural changes from normal mouse karyotype as well as heteroploidy. Prominent among the structural changes were the formation of dicentric, metacentric, subtelocentric, and minute chromosomes. Chu and Giles (1957) found similar changes in human cell strain HeLa; and Chu, Sanford and Earle (1958) recorded ring chromosomes in certain mouse lines in addition to other anomalies. Moreover, in all the strains studied, none was found to be cytologically homogeneous.

Strain L (Earle, 1943) is probably one of the oldest existing cell lines. Since Sanford and her associates (1948) isolated a single L-cell to produce a clone, practically all the laboratories working with tissue culture materials have carried Clone 929 one time or another. A number of laboratories still employ Clone 929 as one of their principal materials. It is conceivable that workers in different laboratories have used different environments, such as nutrition, temperature, mechanical and chemical handling, toxic substances, etc., to cultivate the cells, either consciously or unconsciously. These treatments, or sometimes abuses, would serve as selection forces to influence the composition of cell populations, and genotypes unfit for one environment may gain popularity when conditions alter. Thus even if the original Clone 929 was a relatively homogeneous population, the cytological picture of the sublines carried in various laboratories may well be different. In fact Hsu and Klatt (1958) already reported a few such cases.

The present communication presents observational results from a survey of twelve L-lines to substantiate the concept of genetic polymorphism.

MATERIAL AND METHODS

All the cell strains employed in this study were sublines of Clone 929, Strain L. They were kindly supplied by various investigators to whom we are extremely grateful. The following list summarizes the origin of these strains:

Clone 2071, from Dr. Wilton R. Earle, National Cancer Institute, Bethesda, Maryland.

Clone A929, from Dr. Charity Waymouth, Roscoe Jackson Memorial Laboratory, Bar Harbor, Maine.

Clone 929, from Dr. Donald J. Merchant, University of Michigan, Ann Arbor, Michigan.

L₁, L₂, L₃, L_C, L_B, L_{OB}, and L_E, all the preceding strains from Dr. Sergey

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Fedoroff, University of Saskatchewan, Saskatoon, Canada. The origin of these lines will be described by Fedoroff and Cook (in press). Briefly, Strains L_B and L_{OB} originated from Strain L_1 , and Strains L_C and L_E were derived from L_B .

L-P55, from Dr. Charles M. Pomerat, The University of Texas Medical Branch, Galveston, Texas.

L-P55-N2, a subline of LP-55, established in our laboratory.

The cell lines, upon arrival, were subcultured in Blake bottles with 10% horse serum in Eagle's basic medium. Cytological preparations were made as soon as practicable so as to minimize possible changes in genotype induced by our system of culture handling. The technique of preparing slides was the colchicine-hypotonic squash. For each strain 50 cells were carefully studied in regard to their chromosomal composition.

RESULTS AND DISCUSSION

As has been reported, Strains L-P55 and L-P55-N2 possessed one or two dicentric chromosomes per cell. It was found out later that one of the constrictions was in reality a deep secondary constriction, so that the chromosome was a long subtelocentric. Regardless of the nature of the constrictions, the chromosome was still an excellent marker and will be henceforth referred to as the D-chromosome (fig. 1). This element was also observed in Strains L_2 , L_3 , L_{OB} , L_B , and the stock from Michigan University. In the Michigan strain, however, a number of the cells contained a special chromosome with a median centric constriction and two subterminal secondary constrictions (fig. 2). Probably this chromosome (T-chromosome) was a derivative of the D-chromosome. The two types of chromosome never appeared in one cell, but cells with two D's or two T's have repeatedly been observed in this line. The proportion of cells containing D-chromosome and those containing T-chromosome was approximately 4:1.

As has been mentioned previously, Strain L_B and L_{OB} , both of which contained the D-chromosome, were actually substrains of L_1 . Yet the D-element was not observed in L_1 . It is very unlikely that rearrangement which had produced the D-chromosome could be formed repeatedly, so that Strain L_1 either had contained D-chromosome in its past or still contained such chromosome at a very low frequency when the sample was taken. In either case it suggests that both L_B and L_{OB} were not clone derivatives.

Minute chromosomes in Clone 2071 from National Cancer Institute were also found in a number of strains. There were at least two types of minutes, one slightly larger than the other, and both may appear in a single cell. In the strain from the Roscoe Jackson Laboratory many cells contained a medium sized telocentric chromosome that had a secondary constriction lying close to the centromere. This chromosome was also present in several lines. It is of course not possible to establish identity of two morphologically similar marker chromosomes present in two different strains, but it is probably not unsafe to conclude that most marker chromosomes were not characteristic of any particular strain, but were shared by a number of strains. The only obvious exception was the T-chromosome of the Merchant line which has not been found elsewhere.

Quantitatively, none of the strains analyzed was identical with another. Table

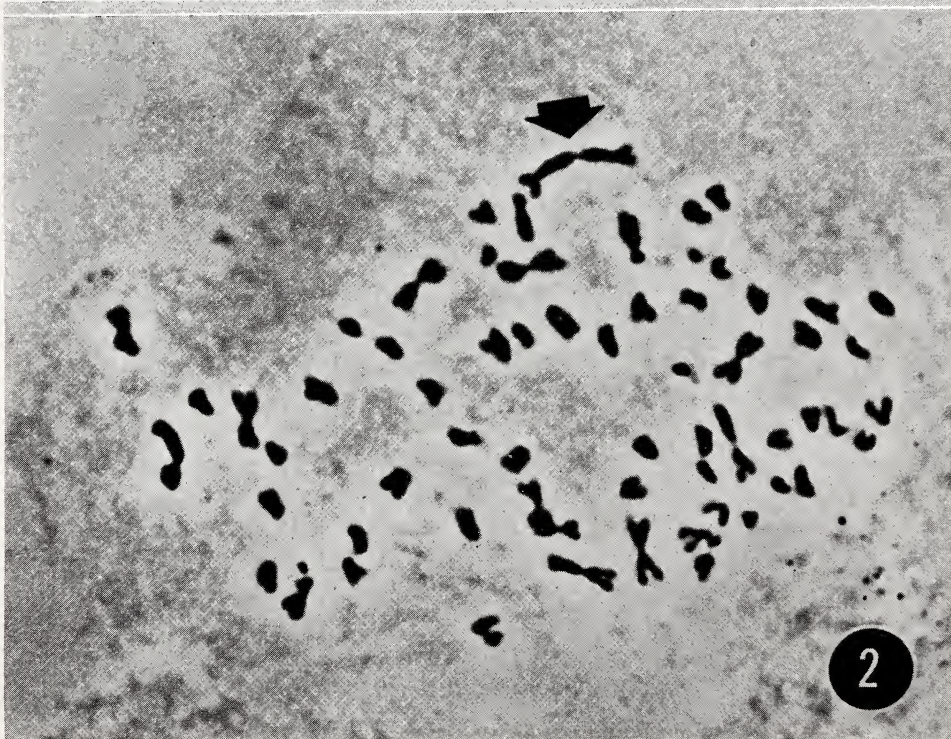
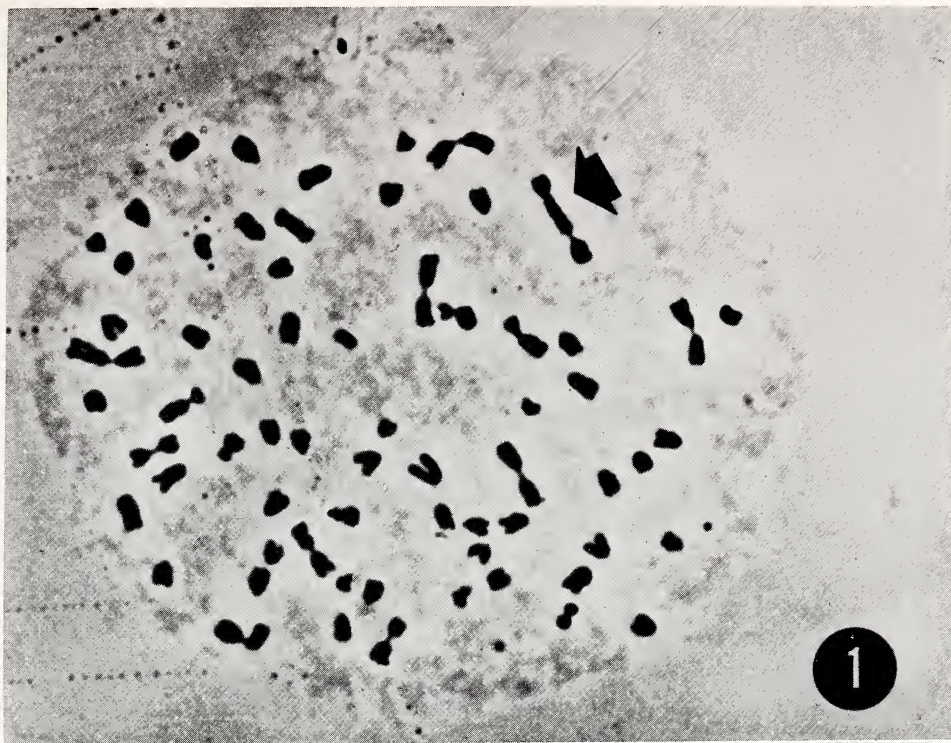


FIG. 1. A cell from the Michigan University subline showing the D-chromosome (arrow).
FIG. 2. A cell from the Michigan University subline showing the T-chromosome (arrow).

I presents data obtained from twelve strains showing mean values of total chromosome number, of number of metacentrics, subtelocentrics, D-chromosomes, and minutes. The total chromosome number was rather similar among all the strains ranging from the lowest mean value of 67 (Waymouth) to the highest of 73 (2071), a difference of six chromosomes. The number of metacentric chromosomes per cell, however, varied more significantly. The lowest mean value found was 12.5 (L-P55-N2), and the highest, 21.5 (L_B), a difference of nine chromosomes. Variation in the number of subtelocentrics was relatively small, but that of the D-chromosome and of the minutes ranged from completely absent to at least one per cell.

It should be added that the strains also differed in the frequency of high polyploids. In most strains high polyploids occupied less than 1 per cent of the population, but in strain L_1 the value reached as high as 24.8 per cent.

In Table 1 an attempt was also made to calculate the percentage of biarmed chromosomes per cell. The values ranged from approximately 24 per cent to 32 per cent. It is understood that these chromosomes were not the only ones that had structural alterations.

Our results substantiate the conclusion that most cell populations in tissue culture are genetically polymorphic. Of special interest is the fact that all the

TABLE 1

Mean values and standard deviations of chromosome types in 12 sublines of Strain L, Clone 929

Strain Designation	Source of Material	Total Chromosome Number	Number of Metacentrics	Number of Subtelocentrics	Number of D-Chromosomes	Number of Minutes	Percent Biarmed Chromosomes
L_C	Fedoroff	71.66 $\pm$ 1.85	13.68 $\pm$ 1.18	3.58 $\pm$ 0.61	0.00	1.10 $\pm$ 0.63	24.21
L_3	Fedoroff	72.74 $\pm$ 1.82	15.00 $\pm$ 1.20	3.24 $\pm$ 0.82	0.02 $\pm$ 0.14	0.44 $\pm$ 0.54	25.13
L_{OB}	Fedoroff	68.14 $\pm$ 3.00	14.18 $\pm$ 1.66	3.28 $\pm$ 0.78	0.14 $\pm$ 0.35	0.86 $\pm$ 0.70	26.00
L_1	Fedoroff	67.96 $\pm$ 1.93	14.68 $\pm$ 1.30	1.42 $\pm$ 0.73	0.00	0.38 $\pm$ 0.49	23.71
L_2	Fedoroff	67.30 $\pm$ 2.38	13.50 $\pm$ 1.61	2.84 $\pm$ 0.91	0.80 $\pm$ 0.45	0.00	27.86
L_B	Fedoroff	67.48 $\pm$ 2.91	21.52 $\pm$ 2.71	2.06 $\pm$ 0.55	0.06 $\pm$ 0.24	0.60 $\pm$ 0.76	35.11
L_E	Fedoroff	71.80 $\pm$ 1.09	14.56 $\pm$ 1.37	2.68 $\pm$ 0.75	0.00	0.68 $\pm$ 0.54	23.01
$L(M)$	Merchant	67.36 $\pm$ 3.28	13.44 $\pm$ 1.86	2.24 $\pm$ 0.74	0.44 $\pm$ 0.50*	1.06 $\pm$ 0.82	23.97
$L(W)$	Waymouth	67.30 $\pm$ 2.89	18.18 $\pm$ 1.45	3.76 $\pm$ 1.06	0.00	0.22 $\pm$ 0.46	32.68
2071	Earle	73.06 $\pm$ 1.70	17.68 $\pm$ 1.39	3.74 $\pm$ 0.75	0.00	0.38 $\pm$ 0.53	29.34
$L-P55$	Pomerat	67.88 $\pm$ 1.81	12.94 $\pm$ 1.32	3.78 $\pm$ 0.86	1.20 $\pm$ 0.61	0.00	25.42
$L-P55-N2$	Hsu	67.86 $\pm$ 1.87	12.50 $\pm$ 1.02	2.06 $\pm$ 0.37	1.60 $\pm$ 0.49	0.00	23.83

* T-chromosomes included in this category.

lines reported here were derivatives of a single cell. Since some marker chromosomes were found in different laboratories which had had no direct contact with one another prior to the time when the cultures were shipped to this laboratory, indication is therefore strong that these chromosomes were present in the original population of Clone 929 in the National Cancer Institute. Perhaps each was present at a relatively low frequency but various new environments facilitated the selection of special genomes. The populations of somatic cells *in vitro* are thus analogous to the populations of *Drosophila* in the well-known population cages.

It should also be borne in mind that each line was represented by only 50 cells. Variation in genotype should indeed be much greater in each population than indicated in Table I. Some genotypes may be at a very low frequency and can be brought to distinction only when the existing superior genomes are not fit for survival. Indeed, experimentally produced changes in genomes have been obtained in Strain L-P55 when the populations were subjected to media containing various sugars to replace glucose (Hsu and Kellogg, in press). In L-P55, the lowest chromosome number per cell registered was 63, and such cells occupied less than 5 per cent of the population. In the special "sugar" lines a large proportion of cells showed chromosome numbers in the vicinity of 63, with the lowest number registered as 59.

Recently Puck (1958) and Tjio and Puck (1958) claimed that some samples of serum used in preparing growth medium for *in vitro* cells contain toxic substances which might induce mitotic anomalies and cause cytological heterogeneity. Application of their procedure may enable investigators to maintain relatively homogeneous populations as experimental materials.

SUMMARY

Twelve sublines of Strain L, Clone 929 were analyzed in regard to their total chromosome number per cell, numbers of metacentric and subtelocentric chromosomes, D-chromosomes, and minute chromosomes. Statistically none of the lines was identical with another, yet marker chromosomes were found in different lines which had never had contact with one another. This indicates that the marker chromosomes must have a long history and they might all have been present in the original 929 clone in the National Cancer Institute before stocks were distributed. Different genotypes developed in different laboratories when conditions favored their survival.

EPILOGUE

It has been over seven years since I left Professor Patterson's laboratory and took a seemingly completely different field of biological research from my previous interest in *Drosophila* genetics. The present article constitutes the eleventh paper on the topic of mammalian chromosomes *in vitro*, but it is interesting to note from this study that a bottle of mammalian cells may also be excellent material for studying population and evolutionary genetics. One can never escape from the influence of such a stimulating teacher.

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Heterochromatic Control of Position-Effect Variegation in *Drosophila*¹

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It is particularly fitting that a discussion of somatic variegation be included in the volume honoring Professor Patterson's eightieth birthday. He was not only among the first of the *Drosophila* geneticists (Patterson 1928) to become interested in the genetics of somatic tissue but he picked this topic to initiate his brilliant series of genetic investigations. His vision is attested to by the fact that now, thirty years later, this field is at the forefront of genetic research and the subject of a recent symposium (Hollaender 1958).

The subject of genetically controlled variegation is intriguing to a student of developmental biology because of its relevance to the problem of differentiation of cellular function. The variegation caused by position effect is of signal interest in this connection because its expression can be altered by a host of inherited factors as well as by certain environmental agents. The work being reported is only an exploratory effort designed to uncover some of the complicating factors involved in V-type position effects. Novel types of genetic control of variegation were discovered during this investigation which may play an important role in cellular differentiation. They also prove to be a source of embarrassing variability if their presence is not recognized; a criticism which is applicable to certain of the experiments being reported herein.

These experiments were initiated with the thought in mind that since extra heterochromatin suppresses white variegation in *Drosophila melanogaster*, an opportunity was provided to determine if there were separable units of suppressing activity within the heterochromatin of the Y chromosome. In other words, could heterochromatin be subdivided into functional units like euchromatic chromosome regions? Different fragments of Y chromosomes, inserted into the genome of three different types of flies, were used to test this notion. The end effect, their influence on the expression of variegation, was measured both visually and chromatographically. The latter procedure provided some evidence on the chemical differences between pigmentation in variegated, white, and wild-type eyes.

MATERIAL AND METHODS

The rearrangement used to produce the white-variegated eyes was derived from N²⁶⁴⁻⁵⁸ (Demerec 1940) which is a transportation of the white region, in reversed order, into the heterochromatin of 3L. It was carried as a duplication, Dp(w^m), the white region of the X chromosome being structurally normal but carrying the w allele. At the beginning of the experiments (1956) it appeared that the duplication was lethal when homozygous in either males or females.

¹ Work performed under contract No. AT(11-1)-431 for the Atomic Energy Commission.

However, in the spring of 1957 (after co-isogenic stocks had been made) viable and fertile females which were homozygous for $Dp(w^m)$ were discovered in the stock cultures. It has been conclusively established that homozygous males, if viable, are sterile; probably they are not viable.

Most of the Y fragments utilized originated from exchange between an inverted X chromosome and a normal Y, or as products of exchange between $X \cdot Y^F$ and a free Y chromosome, or from spontaneous or induced breakage of the $sc^s \cdot Y:bw^+$ chromosome arising in experiments previously reported (Baker, 1955, 1957). A brief description of each of the Y fragments follows:

$Y^{cL}:bb^+$. Arose from X-irradiation (1500r) of sperm bearing the $sc^s \cdot Y:bw^+$ chromosome. It is a moderate size ring-Y similar in size to the three other Y^{cL} 's to be described. (See Figure 7.)

$Y^s:y^+ bb^+-7$. Induced by X-irradiation of $sc^s \cdot Y:bw^+$ sperm (1750r). Cytologically it appears as a small single-armed chromosome about twice the size of chromosome 4.

Y^s . A small two-armed chromosome.

$Y^{cL}-14$. A ring chromosome that arose from X-irradiation (451r) of $sc^s \cdot Y:bw^+$ sperm.

$Y^s:bb^+-8$. Spontaneously arose in $X^{c1}/sc^s \cdot Y:bw^+$ males. It is a small two-armed chromosome.

$Y^s:y^+ bb^+-6$. A two-armed chromosome produced by X-irradiating $sc^s \cdot Y:bw^+$ sperm with 1750r.

$Y^{cS}:bw^+ bb^+$. A ring chromosome arising from $sc^s \cdot Y:bw^+$ sperm treated with 451r of X rays.

$Y^{cL}-15$. A ring chromosome spontaneously arising in $X^{c1}/sc^s \cdot Y:bw^+$ males.

$Y^s \cdot Y^s \#2$. A two-armed chromosome of moderate size formed by exchange between an $X \cdot Y^s$ chromosome and the $sc^s \cdot Y$. (see Muller 1948).

$sc^{v1} \cdot Y^s$. A small two-armed chromosome formed by exchange between a normal Y and the sc^{v1} inverted-X chromosome. (Muller 1948).

Y^{cL} . A ring chromosome which arose in the crosses giving rise to the $Y^s \cdot Y^s \#2$ chromosome. (Muller 1948).

Y^L-13 . A large acrocentric spontaneously arising in $X^{c1}/sc^s \cdot Y:bw^+$ males.

$Y^s:y^+ bb^+-5$. This large acrocentric arose spontaneously in $X^{c1}/sc^s \cdot Y:bw^+$ males.

Y. The Y chromosome from the M-5; Cy; Ubx^{130}/Xa stock. (see Lewis, 1952, for a description of Ubx^{130} .)

$sc^{s1} \cdot Y^L \#2$. This large acrocentric chromosome arose by exchange between a free Y and the distal end of the Muller-5 chromosome (Parker and McCrone 1958).

The study was designed to determine the effect on variegation of these 15 different Y chromosomes in three different genotypes resulting from the matings listed below:

$Y^{sw} y \cdot Y^L y^+ / Y; Dp(w^m) / + \delta \delta \times yw / Y^F \text{ } \bar{y} \bar{y}$ which produces

(1) $Y^{sw} y \cdot Y^L y^+ / Y^F; Dp(w^m) / +$ males.

$X \cdot Y^F / Y^F \delta \delta \times yw / yw; Dp(w^m) / + \bar{y} \bar{y}$ which produces

(2) $yw / Y^F; Dp(w^m) / +$ males.

$X \cdot Y^{-F}/Y^F \delta \delta \times yw/Y; Dp(w^m)/+ \text{♀} \text{♀}$ which produces

(3) $yw/Y^F; Dp(w^m)/+$ females.

(The general notation $X \cdot Y^{-F}$ designates an X chromosome to which is attached a Y that is lacking the fertility factors present in the free fragment; e.g. $X \cdot Y^S/Y^L$ or $X \cdot Y^L/Y^S$.)

It is well known that there are numerous genetic modifiers of variegation; therefore, if a critical study was to be made of the effects of the Y fragments *per se*, co-isogenicity of the stocks used was a necessary prerequisite. This involved the synthesis of 20 different stocks that were as nearly co-isogenic as possible within the limits of the experimental technique employed. It is not feasible to present the detailed breeding scheme which gave rise to these co-isogenic stocks; however, a schematic diagram outlining the protocol used is shown in figure 1. Each block in this diagram represents a stock and within the block is given the genotype of each chromosome in the order (from the left); the X and Y, the 2nd, and the 3rd. The subscripts to the chromosomes (a, b, c, etc.) indicate homologous chromosomes that need not be identical in their genetic composition. Certain limitations of this scheme should be noted. The Y chromosome in all cases comes from the Muller-5; Cy; Ubx¹³⁰/Xa stock but no effort was made to insure that the Y in all strains came from a single Y of the original stock. No control over the heterogeneity of the 4th chromosome was attempted and, of course, the Cy and Ubx¹³⁰ inversions do not completely eliminate the recovery of crossover products providing an additional unmeasured source of variability. Finally, there is free exchange between the third chromosome bearing the duplication and its homolog. This may be an influential factor in causing variability since the remainder of

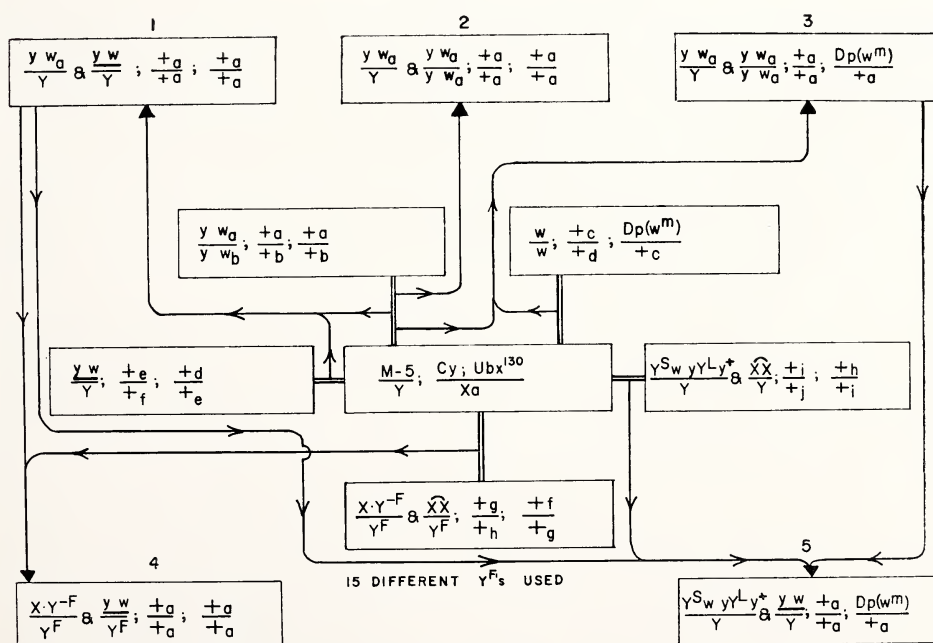


FIG. 1. The breeding scheme for synthesis of the coisogenic stocks.

the chromosome 3 bearing the duplication was not made co-isogenic with the normal 3 from which the other stocks were derived.

The matings in the isogenization scheme were so scheduled that stock #3, stock #5, and most of the 15 different stock #4's were synthesized at about the same time. (These are the stocks necessary to produce the three different genotypes of flies in which variegation was examined.) This enabled the study to be made shortly after isogenization was completed, thus reducing the effect of newly arisen modifiers. After the stocks were synthesized, no controlled breeding pattern was utilized (e.g., brother-sister matings), and the heterozygosity of the duplication was not insured by outcrossing. At that time we did not suspect that flies homozygous for the duplication would rather suddenly become viable nor did we suspect that the homo-vs. heterozygosity of a mother would influence the occurrence and extent of variegation in her heterozygous offspring (Spofford, 1958, 1959).

There is one other potential source of genetic variability that was desirable to control. If nondisjunction of sex chromosomes takes place during the breeding scheme, then an individual fly might contain a Y chromosome in addition to the expected Y-fragment. Or, similarly, two Y-fragments might occur in a fly where only one is anticipated. Since the effect on variegation of different Y fragments was the main purpose of the study, tests had to be made to determine the presence of extra Y's or extra Y^F's. This entailed making a progeny test of each of the individuals scored for variegation. The labor involved when the Y^F did not have a visible marker was enormous and the results, as to be expected, were only partially successful. When the flies to be scored for variegation had eclosed, they were mated, in general, according to the following schemes:

- (1) Y^sw y·Y^Ly⁺/Y^F; Dp(w^m)/+ males × X·Y^{-F}/w females.

If the white male offspring from this cross were sterile, it could be assumed that no free complete Y was present in their father. The fertility of the other class of F₁ male offspring assured the presence of at least one Y^F in the father. The white female offspring were crossed to wild-type males and their offspring examined. If no wild-type males appeared, then their mothers, and therefore the original male parent, did not contain an extra Y^F. For if an extra Y^F had been present in the male, some of his white daughters would receive this chromosome and by secondary nondisjunction would form a few wild-type sons when crossed to wild-type males.

(2) yw/Y^F; Dp(w^m)/+ males were crossed to any type of virgin female to see if they were fertile. Since most of the Y^F's contained either the Y^L or the Y^S fertility factors but not both, this cross tested for the presence of an extra complete Y. Of course, no test can be made for the possibilities that more than one Y^F or no Y^F existed in these sterile males.

(3) yw/Y^F; Dp(w^m)/+ females × y B·Y^{-F}/Y^F males. Fertility of the F₁ males indicates the presence of at least one Y^F in the mother. These F₁ males were crossed to y f females and the resulting sons were checked for fertility, indicating the presence or absence of a complete Y in either of the original parents. The resulting daughters, which should be y B·Y^{-F}/y f were mated to wild-type males and any non-yellow males in their progeny would indicate the probable presence of an extra Y^F in either original parent.

It can be seen from these mating schemes that the presence of an extra Y chromosome can be detected with a good deal of assurance; however, detection of an extra fragment depends on securing a sufficient number of offspring to insure the appearance of secondary exceptions. Since within almost every one of the 48 genotypes examined in this study there was sufficient homogeneity in the appearance of the variegated eyes, it was possible to detect aberrant phenotypes produced by extra Y's or extra Y^F's. Progeny tests were successfully completed on many of the individuals exhibiting the "normal" phenotype for each genotype and on at least some of the phenotypically aberrant flies. The latter tests revealed the expression of variegation resulting from an extra Y chromosome. Thus it seemed justified to exclude from the data other similarly aberrant individuals on which progeny tests were not completed or were ambiguous, and likewise to include flies with "normal" expression of variegation in which the progeny tests were unsuccessful.

The males undergoing the progeny tests remained with their mates for three days before they were removed and scored for variegation; females remained five days. Therefore, all measurements of variegation were made on males that were from three to four days of age and females five to six days. Two types of measurements were made: a visual examination of the extent of pigmentation in each eye, and a chromatographic analysis of the pteridines in each individual head. All the visual estimates were made by one of us (J.B.S.). These estimates took into account primarily the size of the area that was pigmented but also, in certain cases, the intensity of pigmentation. If the visual estimates were to be a function of the amount of pigmentation in the eye, the latter consideration was necessary in particular variegated eyes in which only a "tinge" of pigmentation was present. Each eye was graded separately; a value of 0 was assigned to a completely colorless eye and a value of 1 to a totally pigmented eye, with a variegated eye falling in between these values.

After the visual estimates were made, the fly was decapitated and its head immediately squashed on Whatman No. 1 filter paper for chromatographic analysis. On each 15 x 15 cm. sheet of paper, 10 heads with variegated eyes and one head from an Oregon-R male (3-4 days old) were squashed at 1 cm. intervals, 1.5 cm. from the bottom of the paper, following the procedure of Hadorn and Mitchell (1951). The chromatograms were equilibrated by placing in the bottom of the chromatographic jar the aqueous phase of the butanol-acetic solvent (4 butanol : 5 H₂O : 1 glacial acetic acid) and allowing two hours for equilibration at which time the organic phase was layered on top of the aqueous phase until the bottom of the chromatographic paper was immersed in the developing solvent. The front was allowed to ascend for 2½ hours at 23° C., at which time it had reached 12.5 cm. from the line on which the heads were located. The chromatograms were then removed from the jar and allowed to dry at 23° C.

The ultra-violet fluorescence of the substances chromatographed was examined with a "Shannon" lamp and a Corning No. 9863 filter. Two rectangular areas for each head were cut out of the paper for elution of the fluorescent substances they contained. One area 3 x 0.8 cm., included the head and extended vertically to an R_f value of about 0.22. This area contains all of the visible

pigments and fluoresces orange and yellow (Fl-1 and Fl-2, see Hadorn and Mitchell, 1951). For the purposes of this paper, we shall call this the area containing the "yellow" fluorescing substances. The other area was 1.5 x 0.8 cm. with its lower boundary at about $R_f = 0.30$ and its upper boundary at $R_f = 0.42$. This area includes the substances Fl-4A, 4B and 5 and is called by us the area of "blue" fluorescing substances.

The rectangles cut out of the chromatograms were rolled in a loose spiral, each placed in the bottom of a 10 x 75 mm. test tube, and extracted in 1 cc of a 2% glacial acetic acid solution for 2 hours at 23° C. After this period the paper was removed and the fluorescence of the solution measured by means of a Farrand Photofluorometer. A Corning No. 9863 filter was used between the UV-lamp and the cuvette containing the solution being measured. Between the cuvette and the photo tube (in that order) filters Nos. 4308 and 3385 were inserted for the "blue" substances, and Nos. 3385 and 3389 for the "yellow" substances. The standard used was 1 μ gm/ml of anthranilic acid dissolved in 2% acetic acid. Prior to each reading the fluorometer was adjusted to give a galvanometer reading of 5 for 1 ml. of the standard. Thus the units of fluorescence given in this paper are in units of anthranilic acid fluorescence. The experimental readings have each been corrected for fluorescence of the filter paper by subtracting the fluorescent reading of a paper blank whose size and R_f location were the same as for the experimental area being measured. This correction is valid since the photofluorometer has a linear response to concentration of fluorescing substances over the range employed.

RESULTS

Chromatographic Analysis of Variegated Eyes

An examination in ultraviolet light of the chromatograms of single heads containing white, variegated and wild-type eyes reveals, in general, the following features. White eyes show no fluorescence. In variegated eyes, the amount of fluorescence associated with the visible pigments in the "yellow" area is directly related to the amount of pigmentation in the eye; whereas in the "blue" region from variegated eyes there is more fluorescence than in wild-type if the eyes are about half pigmented. Thus, one of the striking features of variegated eyes is their accumulation of certain of the pteridines far in excess of that found in wild-type.

Figure 2 shows the relations between the amount of the "yellow" and the "blue" fluorescent substances, measured photofluorometrically, and our visual estimate of the amount of pigmentation in 55 individual heads of one genotype. This example is atypical only in the fact that this one genotype of males shows a wide variation of expression of variegation in individual flies, all the way from practically white eyes to those showing almost complete pigmentation. The linear relation found between the amount of the "yellow" substances and our visual estimates of the amount of pigmentation supports the claim that these estimates are reasonably accurate assessments of the extent of pigmentation since the "yellow" fluorescence is associated with the visible pigments on the chromatograms. The excessive amount, over wild-type, of the "blue" fluorescing substances is

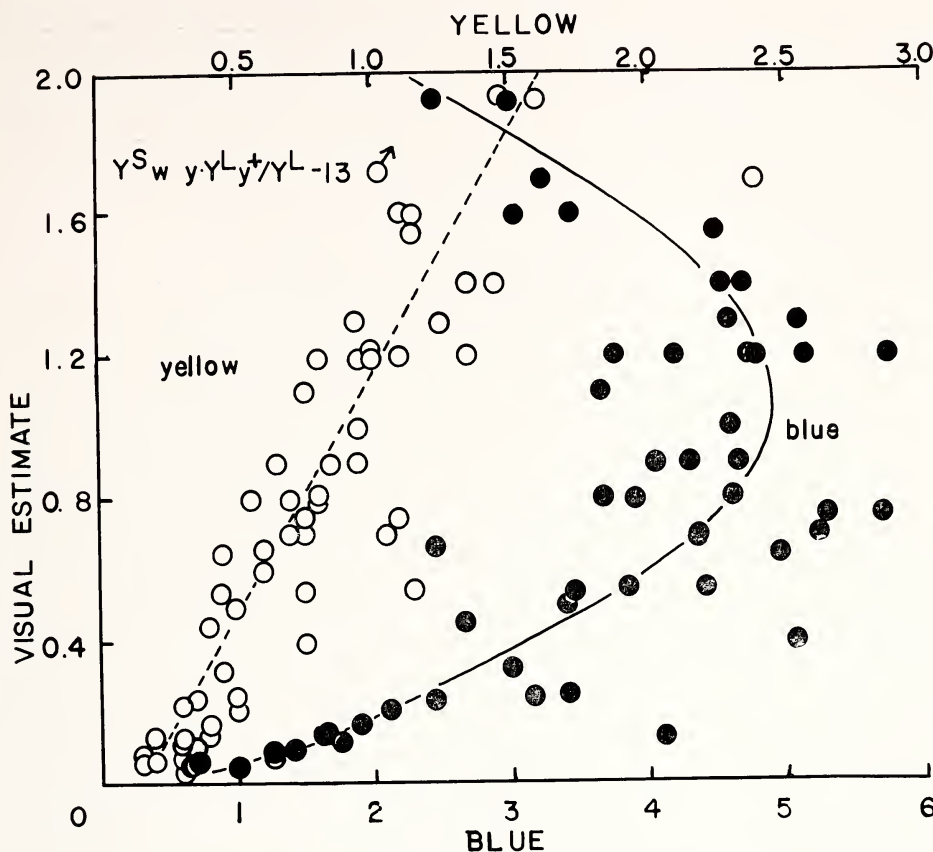


FIG. 2. Relation between the amount of "yellow" and "blue" fluorescence and the extent of pigmentation in $Y^{S_w} y.Y^L y^+ / Y^L -13; Dp(w^m) / +$ males.

clearly evident at intermediate values of variegation. Therefore, it is reasonable to conclude that the pigmented areas of variegated eyes are chemically different (at least quantitatively) from wild-type.

There are three chemical compounds present in the "blue" fluorescing area: Fl-4A which is 2-amino-4-hydroxy-6-(1', 2'-dihydroxypropyl)-pteridine (biopterin); Fl-4B which is 2-amino-4-hydroxypteridine; and the sepia pteridine (Fl-5) which is N⁸-lactyl-7, 8-dihydro-2-amino-4-hydroxypteridine-6-carboxyl acid (see Forrest and Mitchell 1955). The presence of these three compounds in wild-type and variegated eyes was detected by comparing the R_f 's of spots from chromatograms of heads with those made using the pure Fl-4A and Fl-4B, kindly supplied by Dr. Hugh S. Forrest. (The R_f 's for the sepia pteridine were determined from chromatograms of sepia flies.) Four types of chromatograms were used to confirm the identification: one-way ascending in butanol : acetic : H₂O (4:1:5); one-way descending in propanol : 7% NH₄OH (2:1); and two-way, ascending and descending, using these two solvents. For the two-way chromatograms, 20 heads of each genotype were homogenized in 7% NH₄OH, shaken with chloroform, and part of the water-soluble portion spotted on the paper. Since no quanti-

tative comparison of the amount of fluorescence between wild and variegated eyes was made for each of these pteridines, we are not certain as to the extent which each of these substances contributes to the greater fluorescence in the "blue" area in variegated flies. However, it appears that the major portion of the difference is caused by a greater amount of the sepia pteridine in variegated eyes. This would explain the "brownish" appearance of the pigmented areas in many eyes.

Effects of Y-Fragments on Variegation

The main body of data gathered in this study concerns the effect of different fragments of Y chromosomes on the expression of variegation. Four criteria of effect were utilized: a visual estimate of the extent of pigmentation, the amount of "yellow" and "blue" fluorescence, and the penetrance of variegation. The latter criterion deserves further comment. In many of the genotypes, especially with Y fragments that enhance the extent of pigmentation only slightly or not at all, many of the flies will have completely white eyes even though they carry the duplication containing w^m . These cases have been spoken of as those in which the variegation has gone to completion. In the crosses which gave rise to the individuals studied, the duplication was carried in heterozygous condition and in only one of the parents, with the result that half of the offspring would be expected to carry it. If half of the offspring were variegated and half white, then the penetrance is said to be 100%; however, if more offspring were white than variegated the penetrance figure is less than 100%. This, of course, assumes that the third chromosome bearing the duplication is just as likely to be included in the egg nucleus as the normal chromosome 3 and that there is no net influence on viability; reasonable assumptions, but ones for which we have no evidence. In the tables that follow, some of the penetrance figures are given as a range between two percentages. This is done in those cases in which supposedly replicate samples are statistically different from one another in their degree of penetrance.

Let us examine (Table 1) the results of these measurements in $Y^{sw} y \cdot Y^L y^+ / Y^F$; $Dp(w^m) / +$ males. The Y^F 's are arranged in increasing order of effectiveness in enhancing pigmentation (based on the visual estimate). Practically all of the attached-XY males without a fragment have white eyes. Only two out of the estimated 275 males of this genotype showed any pigment and these pigmented areas covered only a few ommatidia. In contrast, the males containing a free fragment all showed a high degree of penetrance even though, with many fragments, the median extent of pigmentation was less than a fifth of the area in each eye. One of the most impressive features of these data is the marked differences in the effectiveness of many of the Y fragments in suppressing variegation; some have very little effect, and others, e.g. $sc^{81} \cdot Y^L$, are just as effective as a complete Y chromosome. It is also of interest to note that the amount of fluorescence in the "blue" region is highest in males with fragment Y^L-13 . These males produce an intermediate value of pigmentation as judged by both the visual estimate and the amount of "yellow" fluorescence. This again attests to the accumulation of certain of the pteridines in variegated eyes.

In table 2 are presented the data obtained from measurements on yw / Y^F ; $Dp(w^m) / +$ females. It is obvious that, with the exception of the last three frag-

ments listed, the eyes of these females have very little pigmentation. In addition, even in those cases where the eyes are heavily pigmented the penetrance is far from complete. Especially noteworthy is the observation that many of the fragments are more effective than the complete Y chromosome in suppressing

TABLE 1
Measurements on $Y^{Sw} y \cdot Y^L y / Y^F$; $Dp(w^m) / +$ males

Y Fragment	Flies Showing Pigmentation		Measurements on Individuals			
	N	Per Cent	Total Number	Median Visual*	Median Blue Fl.†	Median Yellow Fl.†
None	275	1	2	<0.01	...	...
$Y^{cL}; bb+$	199	40-68	50	0.05	0.55	0.05
$Y^S; y+ bb+-7$	178	81	19	0.05	0.65	0.10
Y^S	63	82	34	0.09	1.18	0.28
$Y^{cL}-14$	419	74-92	46	0.10	1.38	0.20
$Y^S; bb+-8$	123	40-97	40	0.10	0.95	0.30
$Y^S; y+ bb+-6$	171	100	53	0.18	0.95	0.40
$Y^{cS}; bw+ bb+$	109	100	45	0.20	1.40	0.38
$Y^{cL}-15$	484	80-100	73	0.24	1.60	0.40
$Y^S; Y^S$	787	78-100	60	0.27	1.70	0.30
$sc^{v1}; Y^S$	302	90-100	38	0.30	1.75	0.33
Y^{cL}	216	74-100	95	0.33	1.78	0.43
Y^L-13	435	94	57	0.75	3.45	0.75
$Y^S; y+ bb+-5$	395	100	50	1.10	1.73	0.85
Y	68	95	52	1.20	2.35	1.00
$sc^{S1}; Y^L$	218	100	47	1.30	2.50	0.95

* White eyes = 0, wild-type eyes = 2.0.

† $1 \mu\text{g/ml}$ of anthranilic acid in elution solvent gives a reading of 5.0.

TABLE 2
Measurements on $\gamma w / Y^F$; $Dp(w^m) / +$ females

Y Fragment	Flies Showing Pigmentation		Measurements on Individuals			
	N	Per Cent	Total Number	Median Visual	Median Blue Fl.	Median Yellow Fl.
None	724	0	..	...	...	...
$Y^S; bb+-8$	618	4	21	0.01	0.10	0.07
$Y^S; y+ bb+-6$	621	5-20	23	0.02	0.26	0.10
$Y^S; Y^S$	800	6	44	0.03	0.35	0.15
Y	242	7-19	27	0.03	0.35	0.20
$Y^{cS}; bw+ bb+$	488	21	50	0.03	0.35	0.25
$Y^{cL}-15$	738	0-25	38	0.04	0.40	0.10
$Y^S; y+ bb+-5$	436	19	57	0.06	0.60	0.20
$Y^{cL}-14$	488	11-33	28	0.05	0.97	0.20
$Y^{cL}; bb+$	433	3	12	0.07	0.15	0.04
$Y^S; y+ bb+-7$	716	10	55	0.08	0.30	0.20
$sc^{v1}; Y^S$	338	4	30	0.16	0.52	0.07
Y^S	172	14-62	33	0.20	1.55	0.35
Y^{cL}	84	62-84	43	1.30	2.90	1.30
Y^L-13	752	51-100	37	1.39	5.45	1.60
$sc^{S1}; Y^L$	620	49-88	36	1.93	3.30	2.20

variegation in this genotype. Once again it is found that females carrying Y^L-13 , which have eyes that are a little over half pigmented, accumulate the "blue" pteridines to a high degree.

The measurements on yw/Y^F ; $Dp(w^m)/+$ males are presented in table 3. The most obvious feature of these males is the small amount of pigmentation in their eyes irrespective of the Y fragments they carry. However, in spite of the small size of the pigmented areas, the penetrance in many cases is moderately high. For example, even though the median extent of pigmentation in males carrying the $Y^S:y^+ bb^+-5$ was only a two-hundredth of the area in each eye, some of the replicates showed 100% penetrance. It is interesting to observe that in these males the complete Y is definitely the most effective enhancer of pigmentation.

The data in the tables previously discussed illustrate quite dramatically that the relative effectiveness of a fragment may be quite different in the three different genotypes of flies in which studies were made. In order to make not only this point clear but also to indicate similarities in patterns of effectiveness of certain of the fragments in the three genotypes, all the data have been summarized in figure 3. The size of the black area in each of the circles in this figure indicates the visually estimated median value for the area of pigmentation in both eyes combined. A glance at this figure will suffice to show that a fragment which is effective in enhancing pigmentation in one genotype may be quite ineffective in one of the other genotypes, or *vice-versa*. This is indicative of a highly complex set of interactions which determine effectiveness of heterochromatin in suppressing variegation. However, certain fragments behave according to particular patterns. For example, Y^{cL} , $sc^{S1}Y^L$, and Y^L-13 are most effective in attached-X females, least effective in normal X males, and have intermediate effectiveness in attached-XY males in as far as the visual estimate of pigmenta-

TABLE 3
Measurements on $y w/Y^F$; $Dp(w^m)/+$ males

Y Fragment	Flies Showing Pigmentation		Measurements on Individuals			
	N	Per Cent	Total Number	Median Visual	Median Blue Fl.	Median Yellow Fl.
None	600	0	..	...	...	...
$Y^S:y^+ bb^+-5$	467	21-100	36	0.01	0.10	0.10
$Y^{cL};bb^+$	494	5	59	0.04	0.25	0.04
Y^{cL}	862	56-95	75	0.06	0.30	0.05
$Y^{cL}-15$	908	3	74	0.06	0.48	0.10
$Y^{cL}-14$	880	26-76	45	0.06	0.60	0.15
$Y^S:y^+ bb^+-6$	639	15-67	65	0.07	0.50	0.20
$Y^S:y^+ bb^+-7$	238	69-94	39	0.07	0.80	0.15
$Y^{cS};bw^+ bb^+$	285	32	89	0.08	0.81	0.17
Y^L-13	638	34-75	43	0.10	1.10	0.15
Y^S	728	14-56	39	0.10	1.15	0.25
$Y^S;bb^+-8$	1000	54-94	100	0.11	0.89	0.26
$sc^{S1}Y^L$	542	52	27	0.14	0.30	0.20
$sc^{V1}Y^S$	294	40-100	37	0.17	2.15	0.30
$Y^S.Y^S$	851	19	45	0.45	2.20	0.30
Y	610	4-62	55	0.65	2.70	0.45

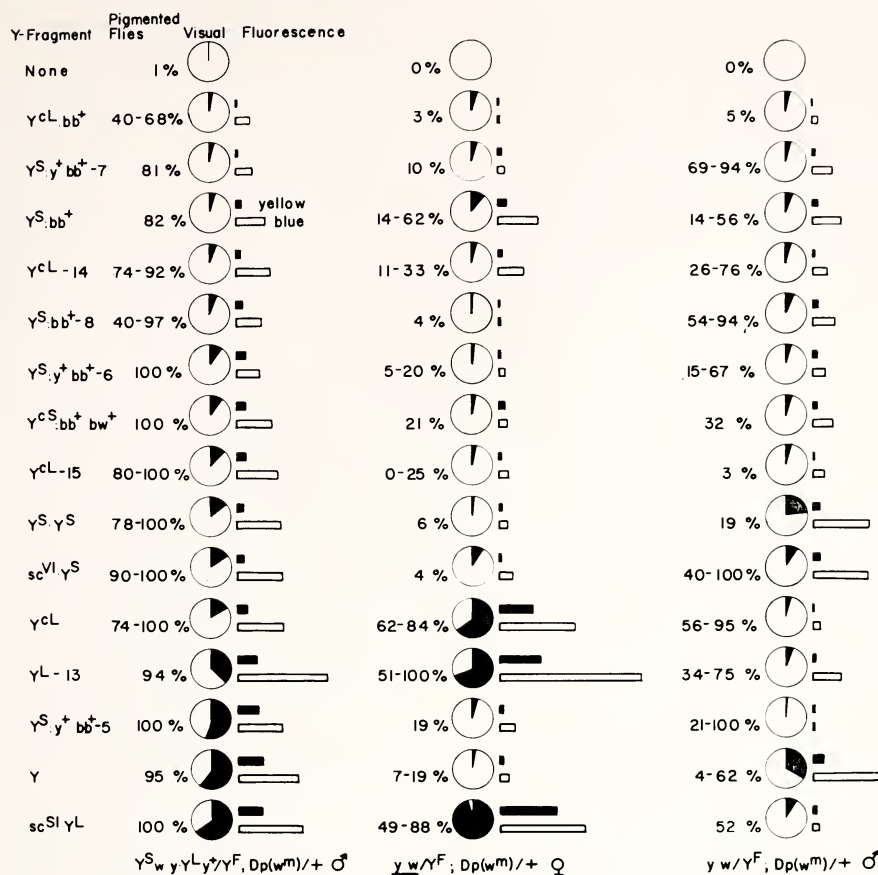


FIG. 3. A summary of the data on how 15 different Y fragments affect the penetrance, the pigmentation, and the amount of certain pteridines in variegated eyes of flies of three genotypes.

tion and the amount of "yellow" and "blue" fluorescing substances are concerned; whereas other fragments, like Y^S.Y^S and Y^S.bb⁺-8 are less effective in attached-X females than in the other two genotypes. These factors are brought out to indicate that not only can many of the Y fragments be differentiated from each other by their effect within a given genotype but also they can be distinguished by their pattern of response among the three genotypes. In particular, note the differences in the four Y^{cL} fragments.

Before proceeding further with an analysis and discussion of these results, it is instructive to learn the extent of variation encountered in the three measurements among individuals of supposedly the same genotype. The data in the previously discussed tables and figure were given in terms of medians. This was felt necessary in view of the fact that in certain of the genotypes and in particular with the "blue" fluorescence measurements there were long tails on the frequency distributions. Histograms showing the distributions for the visual estimates, the "yellow" fluorescence substances, and the "blue" fluorescence are pictured in figures 4, 5 and 6 respectively. Let us consider first the histograms

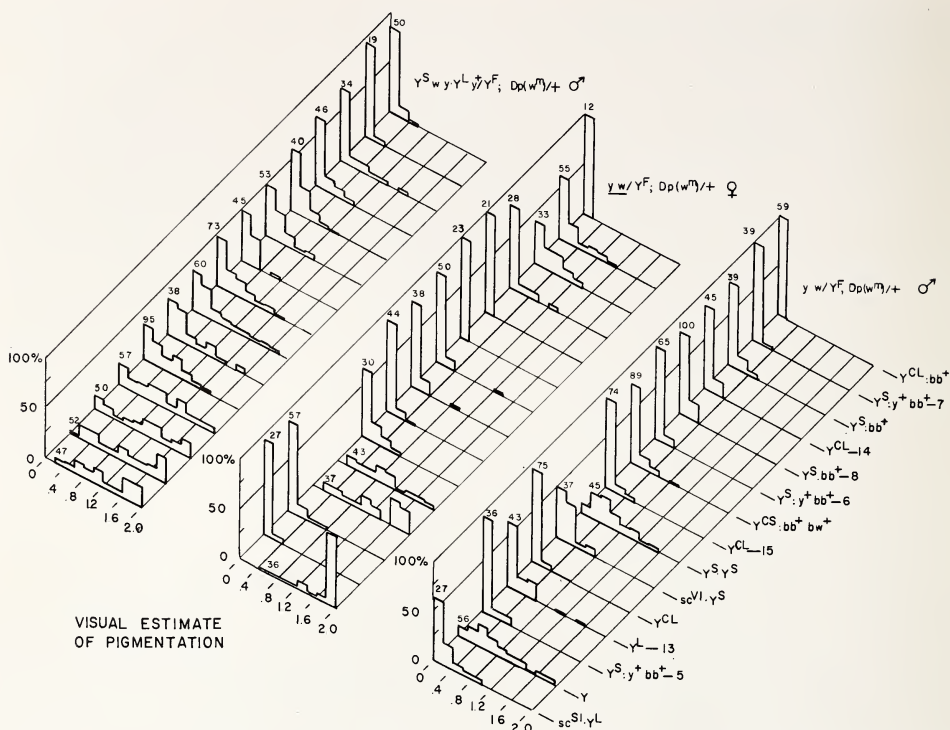


FIG. 4. Frequency histograms of the visual estimates of pigmentation. The figure above each histogram indicates the total number of individuals sampled.

of the visual estimates. After the precautions which had been taken to attain co-isogenicity of the stocks used and the constancy of the environmental conditions under which the flies were reared, it is certainly disturbing to find such wide variation in the extent of variegation as observed, for example, in attached-XY males bearing the Y or the Y^L-13 chromosomes. In these cases not only were flies obtained whose eyes were almost completely pigmented but other individuals had eyes with very little pigment. Part of this variation may be caused by inadequate visual estimates of the intensity of pigmentation since the distributions of the measurements of "yellow" fluorescence (figure 5) were less broad. However, most of the variation is the result of pigmentation differences in the flies *per se* and not caused by errors of estimation or experimental errors in measurements since in many of the genotypes these variations were not observed. A discussion of the possible factors which may be at the basis of this variation will be deferred until later. It is obvious from figure 6 that the variability encountered in the measurements of the "blue" fluorescence is much greater than that observed in the "yellow" fluorescence or with the visual estimates. In general, there is little variability in the amount of "blue" fluorescence when there is very little eye pigmentation as estimated either visually or by "yellow" fluorescence. However, in eyes with moderate amounts of pigmentation the value of "blue" fluorescence varies greatly (see figure 2). This would not appear to be caused by the instability of these pteridines during chromatography since in many instances most

of the heads of one genotype were chromatographed simultaneously under the same conditions. It seems more likely that either these pteridines accumulate under some sort of threshold conditions or that after accumulation they are rapidly used in synthesis and converted to other compounds.

Let us return now to the main finding of this study: many of the Y fragments have radically different potencies in enhancing pigmentation in variegated eyes. The question arises as to whether the effectiveness of a fragment is positively correlated with the amount of heterochromatin it contains, or whether there is evidence of regions of heterochromatin which are more functionally active than other regions. One way of measuring the amount of heterochromatin is by cytological study of the fragments. Figure 7 contains a series of photomicrographs of the Y fragments as seen in aceto-orcein smears of the ventral ganglion of larvae. It can be seen from this figure that the four ring fragments which contain only the Y^L fertility factors are about the same size. Yet we noted that they had quite different effects on the extent of variegation. For example, consider the amount of fluorescence in the "yellow" area of the chromatograms of attached-XY males. Y^{cL-15} produces over six times as much "yellow" fluorescence as does Y^{cL-14} , which in turn has twice the amount present in $Y^{cL:bb^+}$ males. In attached-X females, Y^{cL} produces over six times as much "yellow" fluorescence as does any of the other ring- Y^L fragments. Consider also the fragment $Y^S:y^+bb^+-6$ which, as can be seen in figure 7f, has two arms and contains the nucleolus

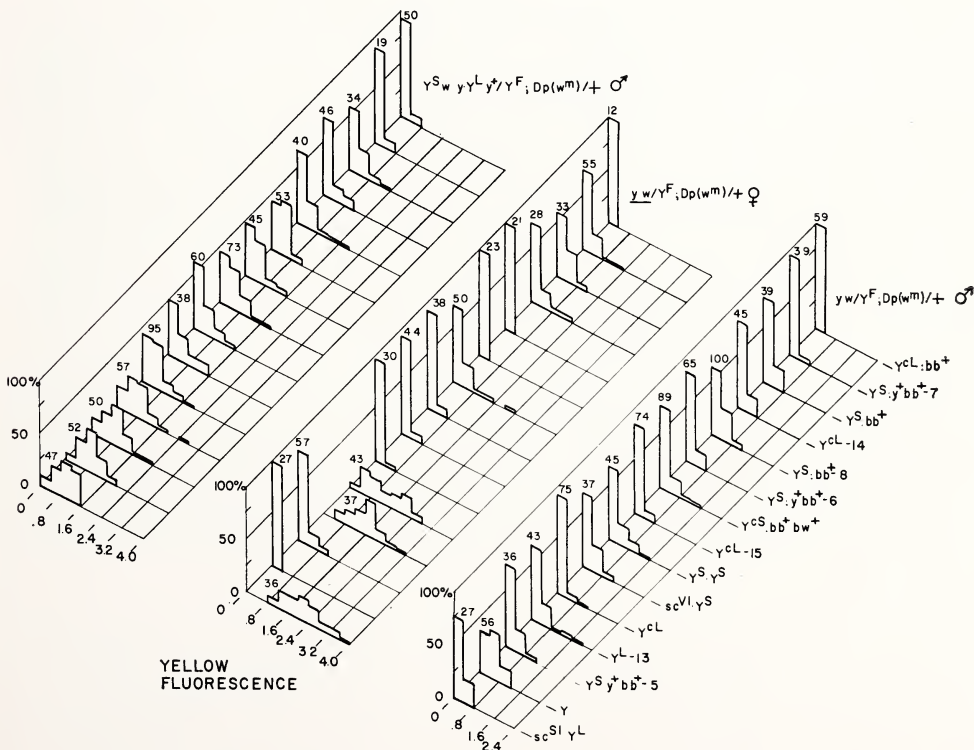


FIG. 5. Frequency histograms of the amount of the "yellow" fluorescing substances.

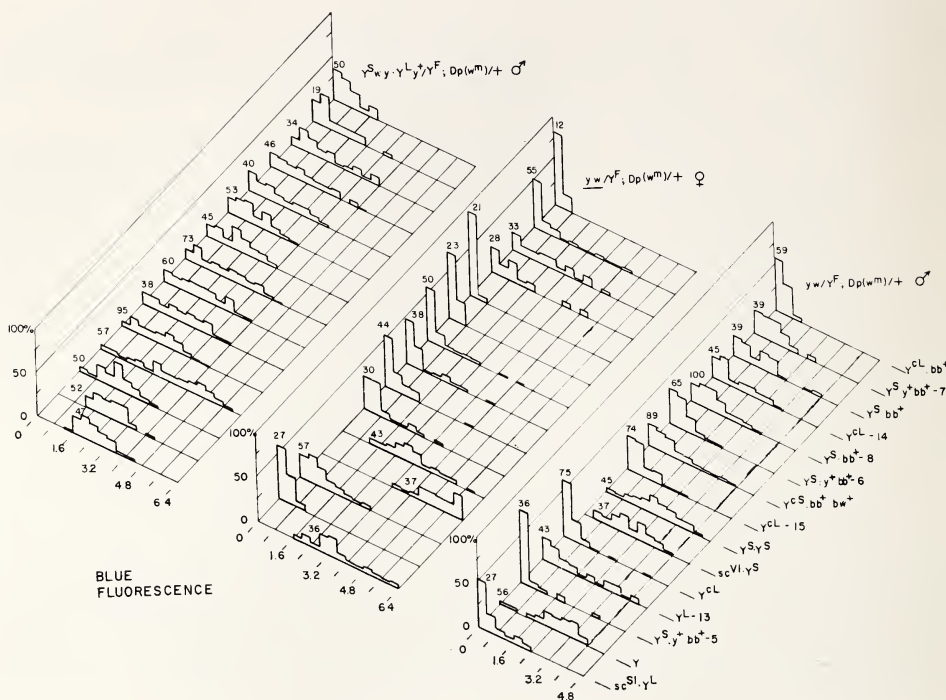


FIG. 6. Frequency histograms of the amount of the "blue" fluorescing substances.

organizer. This moderate-sized fragment is very ineffective in enhancing pigmentation in all three of the genotypes studied. One is led to the conclusion that there are regions of heterochromatin which are functionally more active than other regions, thus suppression of variegation is not entirely a matter of the quantity of heterochromatin. In fact, the observation that in attached-X females and in attached-XY males some of the fragments are more effective than even a complete Y leads one to believe that there may be some regions of heterochromatin that act as suppressors of the regions that are actively involved in promoting pigmentation.

DISCUSSION

The variegated-type of position effect has been studied in *Drosophila* for three decades. In spite of the impressive array of facts that have been gleaned, none of the testable hypotheses that have been proposed has withstood empirical examination. Our experiments only add complexity, not clarity, to the issues involved, but one hopes that the marshaling of new facts will lead eventually to a general explanation.

Since practically all of the V-type position effects involve a euchromatic-heterochromatic chromosomal rearrangement and since heterochromatic addition to, or subtraction from, the genome changes the expression of the gene concerned, the functions of heterochromatin within a cell would appear to be the key to an explanation. Schultz (1956) has suggested, and he and his co-workers have obtained evidence, that heterochromatin is concerned with nucleic

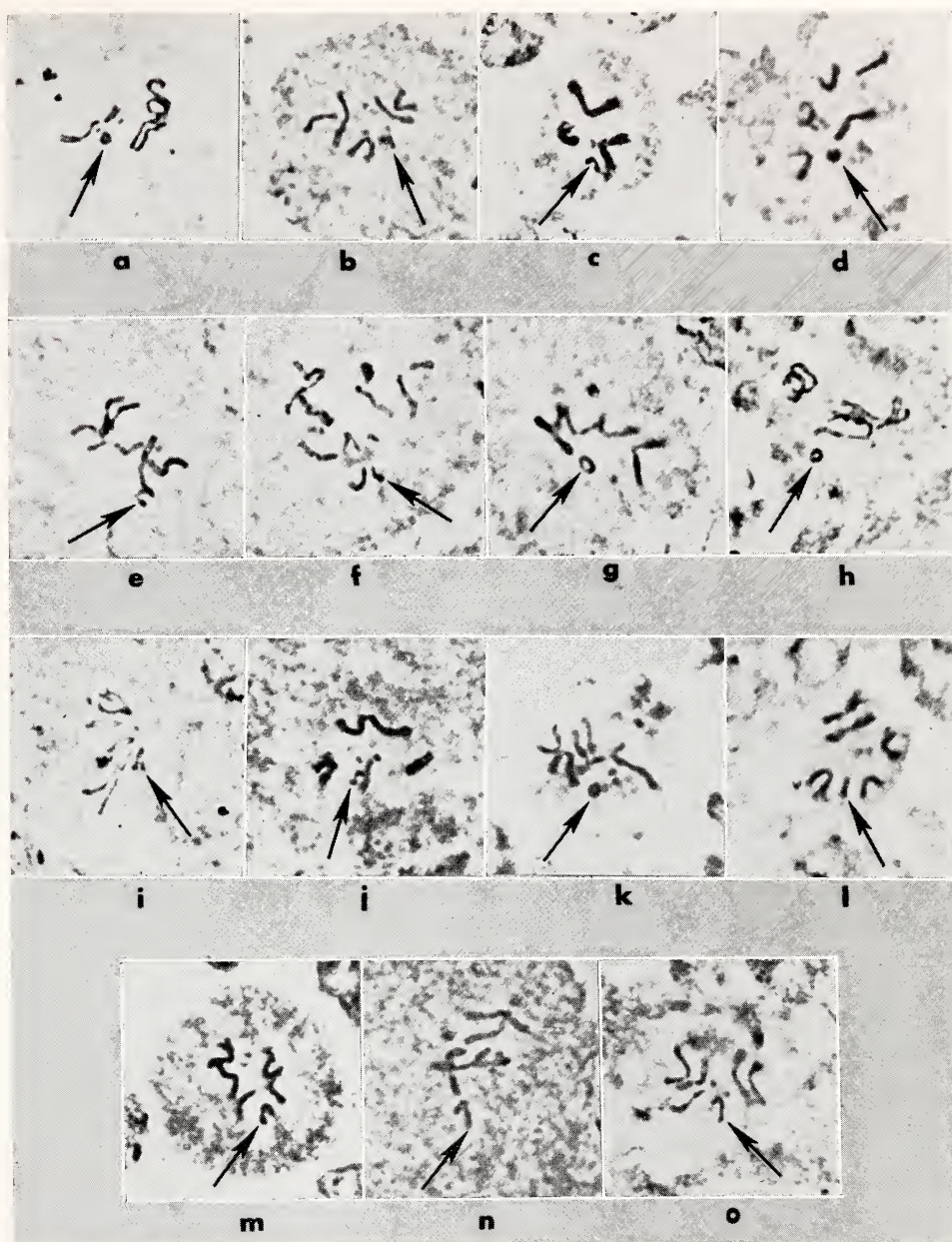


FIG. 7. The cytological structure of the different Y fragments.

(a) $Y^{cL};bb+/X\cdot X$ female. (b) $Y^S;y+bb+-7/X$ male. (c) $Y^S/X\cdot X$ female. (d) Y^{cL-14}/X male. (e) $Y^S;bb+-8/X\cdot X$ female. (f) $Y^S;y+bb+-6/X\cdot X$ female. (g) $Y^{cS};bw+bb+/X\cdot X$ female. (h) $Y^{cL-15}/X\cdot X$ female. (i) $Y^S;Y^S\#2/X\cdot X$ female. (j) $sc^{V1}.Y^S/X$ male. (k) Y^{cL}/X male. (l) $Y^{L-13}/X\cdot X$ female. (m) $Y^S;y+bb+-5/X$ male. (n) Y/X male. (o) $sc^{S1}.Y^L\#2/X$ male.

acid metabolism. The importance of this finding should not be underestimated but its relevance to an explanation of position effect is not clear at this stage of our knowledge. The well-known fact should be recalled that the deletion or addition of large heterochromatic regions has no effect on gene expression of the w^+ locus unless a euchromatic-heterochromatic rearrangement has occurred. One would conclude that functioning of large heterochromatic regions is not essential for normal gene expression. On the other hand, once a break is produced in the heterochromatin and a locus normally situated in euchromatin is juxtaposed to the heterochromatic break, then the gene expression of this locus is altered. There is no evidence to indicate that any deletion of heterochromatin is necessary to produce this effect; an interruption of its continuity is sufficient. Neither the heterochromatic break alone nor the close proximity of a euchromatic gene to heterochromatin *per se* is a sufficient cause for the effect; both of these primary factors must be present for variegation to occur.

The gene concerned must not only be close to the region of interrupted heterochromatin, it must be on the same member of the homologous pair of chromosomes if its action is to be affected. It may not be generally realized that the variegated-type of position effect exhibits, like position pseudoalleles, the cis-trans phenomenon. If one considers the heterochromatic break in the rearrangement causing position effect as a region of mutant heterochromatin (h), then it has been shown that $w^+ h/w h^+$ produces variegated eyes whereas $w^+ h^+/w h$ flies, derived by exchange between the locus and the break point, have normal eyes (Judd 1955). Therefore, the primary action of the disturbed heterochromatin extends linearly along the chromosome with no interaction between homologs. This is also exemplified by the spreading effect (see Lewis 1950 for a review). This barrier between the gene action of each homolog does not necessarily imply a physical barrier between homologous chromosomes. Perhaps the chromosome products of a particular region of one homolog which are released into the cytoplasm retain a linear continuity encompassing part of the disturbed heterochromatin as well as the affected loci. If the effect on gene expression is mediated through synthetic activities occurring in the cytoplasm, and if the chromosome products of each homolog are released independently, then a competent spatial barrier in the cytoplasm may result.

Now if both the primary factors for variegation are present, then its expression and penetrance are secondarily influenced by the addition or subtraction of heterochromatin from the genome. If one supposes that the postulated linear pieces of chromosomal product are RNA, then the demonstrated effect of heterochromatin on nucleic acid metabolism might be at the root of these secondary effects.

In this connection, it is interesting to note that our data indicate that the heterochromatin of the Y chromosome contains regions which are much more effective than others in influencing the extent of pigmentation. In fact, some of the Y fragments are more effective than a complete Y. A study of the nucleic acid metabolism in cells with these fragments might prove illuminating. It should not be forgotten that even these secondary effects on variegation take different directions depending on the type of gene involved in the rearrangement. Extra heterochromatin increases pigmentation and subtraction of heterochromatin

decreases pigmentation in variegated eyes caused by rearrangement involving a gene normally located in euchromatin, like the white locus. However, just the opposite relation is found with position effects of genes normally located in heterochromatin; e.g., the light locus (Schultz 1936). Since we have not as yet conducted studies on the effect of these same Y fragments on variegation at light, we do not know whether the fragments which are most effective in suppressing variegation at white are the same fragments most potent in enhancing light variegation, or *vice-versa*. We may be deluding ourselves in conceiving that the heterochromatin concerned secondarily with variegation has a single function; more than likely, it is multifunctional with spatially separated units.

The secondary factors which affect the expression of variegation are not limited to heterochromatic components of the genome of the individual in question, but extend to the genotypes of the parents (Spofford 1958). The details of these parental effects will be presented in another paper (Spofford, 1959), suffice it to say that their cause is not restricted to the amount or to the suppressing quality of any extra heterochromatin in the parents. For example, the extent of pigmentation in the variegated offspring is dramatically influenced by whether or not the mother was homo- or heterozygous for $Dp(w^m)$. In addition, it is influenced by which parent contributes the duplication and by whether or not the mother is homozygous for a recessive modifier which segregates independently from the duplication. Thus there is an array of hereditary factors, some intrinsic, others not, which mimic one another in their effects on variegation. The environmental temperature has an action similar to the hereditary factors (Gowen and Gay 1933).

It seems clear that unless these secondary factors are under experimental control, the interpretation of data on position-effect variegation may be ambiguous. The secondary factors which were not under control when our studies were initiated were the parental effects just mentioned; in fact, their presence was only discovered during the course of the experiments. One of these factors which could be responsible for a portion of the variation observed between individuals of a given genotype is whether or not the mother was homozygous for the duplication. The duplication was of maternal origin in the X/Y^F males and the $X\cdot X/Y^F$ females. An examination of the data on the extent of pigmentation and "yellow" fluorescence (figures 4 and 5) shows no more intra-genotypic variation among individuals of these two genotypes than in $X\cdot Y/Y^F$ males although the penetrance figures (tables 1, 2 and 3) suggest less variability in the latter males. One can only conclude that an unmeasured portion of the variability in X/Y^F males and $X\cdot X/Y^F$ females should be attributed to this cause.

The parental effect which might make inter-genotypic comparisons ambiguous is the observation that more pigmentation in the variegated eyes is produced if the duplication and the sex chromosome bearing the mutant allele of white are both contributed by the father rather than the mother. In our experiments, the $X\cdot Y/Y^F$ males received these two elements from their father, whereas the X/Y^F males and the $X\cdot X/Y^F$ females received them from their mothers. Therefore, the general increase in pigmentation in $X\cdot Y/Y^F$ males over the other two genotypes may be at least partly the result of this parental effect. However, note that with

four of the fragments Y^S , Y^{cL} , Y^L-13 , and $sc^{s1} \cdot Y^L$, more pigmentation was present in the attached-X females than in attached-XY males.

Finally, the high degree of pigmentation in $X \cdot Y/Y^S: y^+ bb^+-5$ males is undoubtedly the result of the "residual effect" of this fragment. Spofford (1959) has shown that this fragment in attached-X mothers produces more pigmentation in variegated offspring—irrespective of the Y fragment present in the offspring—than several other Y fragments in attached-X mothers.

Two interesting inter-genotypic comparisons should be mentioned. It was noted that the penetrance of variegation was higher in both types of males than in the attached-X females. This observation can be extended to free-X, $y w/y w$; $Dp(w^m)$ females since they also show low penetrance (unpublished data). It appears that penetrance is inversely correlated with the dosage of the mutant allele of white. If these white alleles are amorphs, there is a balance, relating the number of sets of X-chromosome genes to gene expression, which requires more activity of $Dp(w^m)$ in females than in males for pigment formation.

Another inter-genotypic comparison of interest is between the variegation seen in $Y^{sw} \cdot Y^L y^+/O$ males and $y w/Y$ males (see tables 1 and 3). Although the former males contain as much, and probably more, heterochromatin than the latter males, only very rarely do their eyes show any pigment at all and then only just a trace. The X/Y males are quite heavily pigmented. This observation raises again the possibility that a certain amount of structural integrity of the heterochromatin must be retained if its functions in suppressing variegation are to be maintained. Three alternatives are suggested: (1) The Y heterochromatin is subdivided into separate functional units, in so far as their effect on variegation is concerned, and during the synthesis of the attached-XY chromosome (Lindsley and Novitski 1950) some of the potent regions were lost. (2) Irrespective of whether or not the Y heterochromatin is delineated into functional units, the effectiveness of the whole depends on the structural integrity of its parts. (3) The activity of the hypothetical functional units in the Y may be suppressed by a rearrangement which associated them with euchromatin. It would be interesting to see if the $XY^L \cdot Y^S$ chromosome of Parker (1954), derived from detachment of an attached-X with the $sc^s \cdot Y$, shows this same reduced activity towards suppression of variegation. In any event these observations should serve to warn one against the possible pitfalls of using attached-XY chromosomes to increase the amount of active heterochromatin in a genome.

One additional finding of ours deserves further comment. The increased amount of the sepia pteridine in variegated eyes over that found in wild type points to the possibility that steps in the synthesis of pigment are being affected which occur later than the step ostensibly controlled by the white locus. This conclusion is reached because the sepia pteridine is not found in adult flies with white eyes no matter whether they are homozygous for w or $cn bw$ (Hadorn and Mitchell 1951). Since even bw flies do not contain any of this pteridine, one would guess that one of the steps in the synthesis of the red pigments affected by $Dp(w^m)$ follows after the step controlled by the bw locus. One might still retain the one gene-one action hypothesis and explain this dichotomy of action of the white locus by assuming that its primary function is the production of a co-enzyme, or perhaps a polypeptide, which is a component of at least two different

enzymes controlling the synthesis of pigment. One of the enzymes may control a reaction leading to intermediates that are common to both the red and the brown pigments: whereas, the other enzyme might be concerned with the metabolism of the sepia pteridine. Further speculation at this time seems fruitless, but at least the possibility should be kept in mind that the white locus may not be *directly* involved in the synthetic pathways leading toward pigment formation.

The altered gene expression caused by $Dp(w^m)$ is distinguishable from that seen with either the w^a or the w^e mutant alleles. Hadorn and Mitchell, *loc. cit.*, found a reduced value of Fl-4 plus Fl-5 (the sepia pteridine) in these mutants as compared with wild type. This fact in itself suggests that variegation is not the result of somatic mutation but rather altered gene action, often in a clonal pattern. The results of further experiments addressed to this topic will be reported in a subsequent paper.

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SUMMARY

The effect of 15 different fragments of the Y chromosome (Y^F 's), in suppressing position-effect variegation was studied in *Drosophila melanogaster*. The chromosomal rearrangement responsible for the variegation is an insertion of the w^+ locus into the centromeric heterochromatin of 3L and is designated $Dp(w^m)$. The suppressing action of these fragments on the size of the white areas in the variegated eyes was recorded visually and chromatographically in flies of the following genotypes who were otherwise coisogenic: $y w/Y^F$; $Dp(w^m)/+$ males, yw/Y^F ; $Dp(w^m)/+$ attached-X females, and $Y^s w y \cdot Y^L y^+/Y^F$; $Dp(w^m)/+$ attached-XY males.

Many of the Y^F 's were found to differ markedly in their potency; some even more effective than a complete Y. Cytological examination indicates that suppressing activity is not necessarily correlated with the amount of heterochromatin in the fragment. Thus the conclusion is drawn that Y heterochromatin may be subdivided into functional units.

Variegated eyes with an intermediate extent of pigmentation accumulate an amount of the sepia pteridine greatly in excess of that found in wild-type eyes; whereas, white eyes contain none of this compound. This suggests that the action of w^+ which is being altered by position effect occurs at a rather late stage in the synthesis of the red pigments.

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Genetic Studies on the Cardini Group of *Drosophila* in the West Indies

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INTRODUCTION

Geographic isolation is the main prerequisite to the initiation of genetic divergence of sexually reproducing populations. Island populations afford an excellent opportunity to study the effects of isolation, especially when the forms under study can be crossed in the laboratory. The cardini group of *Drosophila*, restricted chiefly to the Neotropical Region, exhibits an interesting distributional pattern in the West Indies, part of which has recently been discussed (Heed, 1957a), and contains an array of forms which can be subjected to laboratory analysis.

The present report deals mainly with the genetic and cytological affinities of the forms inhabiting the Lesser Antilles and Puerto Rico, described below as the dunni subgroup of the cardini group. The series is named after *D. dunni*, described by Townsend and Wheeler (1955) from Puerto Rico. The taxonomy of the series has not been completely worked out and will be reported separately. The geographic origin, stock number and symbol of each of the strains studied is given below; additional collection data are shown in Table 17.

D. acutilabella: St. Petersburg, Fla. (F), 2099.1; Floral City, Fla. (Fl), 2302.12; Cuba (C), 2380.2; Jamaica (J), H137.5; Haiti (H), H135.7.

D. belladunni: Jamaica (Jb), H356.3.

D. dunni subgroup: Puerto Rico (PR), 2327.1; St. Thomas (ST), H253.5; St. Kitts (SK), H126.1; Guadeloupe (GU), H252.7; Martinique (MA), H248.1; St. Lucia (SL), H122.1; Barbados (BA), H247.1; St. Vincent (SV), H240.1; Grenada (GR), H239.6.

Figure 1 illustrates that, in general, each of the islands has its own phenotypically distinguishable population and that the change in abdominal pattern from north to south forms a more or less regular sequence or cline. Such an obvious cline in abdominal pattern is a rare phenomenon in *Drosophila* species. The chain of islands of the Lesser Antilles and Puerto Rico may be exactly superimposed on the state of Florida, for they extend in an arc only 734 miles. The majority of the main islands are separated from one another by 20–30 miles of open water. This affords the isolation necessary to provoke divergence of an otherwise more or less continuous genetic system. The adaptive significance of the darker flies to the south and lighter flies to the north is not known. A study of the microhabitats on the islands in conjunction with an analysis of the inheritance of the abdominal pattern may yield some information on this, for most assuredly the cline is the result of present day selective values superimposed upon the past history of the group. The tests made to date indicate that the inheritance of the abdominal pattern depends on a number of genetic factors, apparently of

unequal effects, and is difficult to analyze. Further tests must be made before a report on this phase of the study is possible.

Another interesting feature of the dunni subgroup which can be briefly mentioned is that the larvae of every island stock exhibit a low rate of cannibalism. Larvae have been observed to feed on other apparently normal larvae (as well as

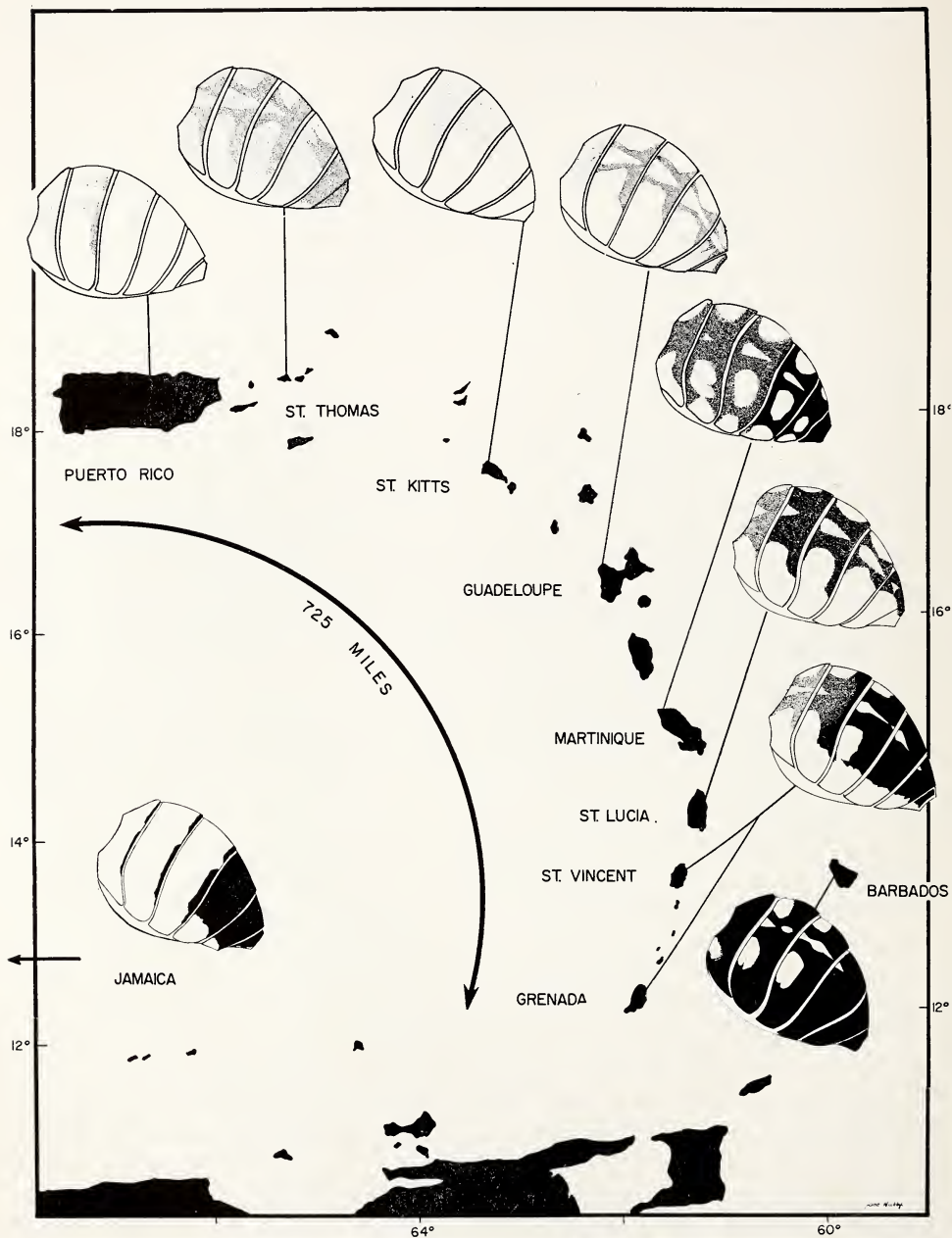


FIG. 1. Abdominal patterns of the dunni subgroup females in the Lesser Antilles and Puerto Rico; the pattern of *belladunni* from Jamaica is also shown.

tumorous larvae, which are fairly frequent) and also to scrape their way into puparia and feed on their contents. This phenomenon has not been previously reported in *Drosophila* and deserves further investigation. It is interesting that the cardini group is one of the groups of *Drosophila* that have skipping larvae. In the case of the dunni subgroup the skipping habit would have a distinct selective advantage, on the assumption that cannibalism *per se* is a detriment to the population. The assumption however may not be correct. Also there is the possibility that the larvae are predatory on larvae of other species. This has not yet been tested.

The following report is divided into four sections. The first two sections briefly describe the distribution of the group on the islands and the collection data as it concerns the dunni subgroup. The purpose is to show that this subgroup is characteristic of the Lesser Antillean and Puerto Rican faunas both in natural and domestic environments. The last two sections concern the hybridization tests, carried out mostly by the senior author, and the cytological analyses, made by the junior author.

TAXONOMY AND DISTRIBUTION

The cardini species group of the subgenus *Drosophila* was established by Sturtevant in 1942 for *Drosophila cardini* Sturtevant, with *similis* Williston, *albirostris* Sturtevant and *metzii* Sturtevant indicated as other possible members. Since that time the following species have been added to the group: *cardinoides* Dobzhansky and Pavan, *neocardini* Streisinger, *polymorpha* Dobzhansky and Pavan, *acutilabella* Stalker, *parthenogenetica* Stalker, *dunni* Townsend and Wheeler, *neomorpha* Heed and Wheeler, *nigrodunni* Heed and Wheeler, and *procardinoides* Frydenberg, while *albirostris* and *metzii* have been placed in the tripunctata group.

Our studies of the forms on the Lesser Antilles and Puerto Rico showing a cline in abdominal pattern show that they constitute a *dunni* subgroup, clearly differing from the remainder of the cardini group. This is a natural subgroup, the members being more closely related to one another than to other members of the group. A distinguishing feature is that nearly every island population has its own distinct phenotype, especially in the abdominal pattern. The arrangement of bristles on the palpi (5-6 long bristles on the antero-lateral margin) and the cannibalistic habits of the larvae are also common to all members of the subgroup.

The members thus far described are *dunni* Townsend and Wheeler (1955) from Puerto Rico, and *nigrodunni* Heed and Wheeler (1957) from Barbados. *Drosophila similis*, described by Williston from St. Vincent, may be conspecific with the form that we have studied from that island.

The new species described below, *Drosophila belladunni* from Jamaica, is not included in the dunni subgroup since it will not cross as well with those forms as they do with one another.

***Drosophila belladunni*, new species**

This new species from Jamaica is sympatric with and very similar in abdominal pattern to *D. acutilabella* Stalker (1953). The bristle arrangement of the

palpi and the male genitalia are more similar, however, to those of the members of the dunni subgroup. There are no good obvious phenotypic characters that will always distinguish the females of these two species in Jamaica; characters that sometimes work are the larger size and more extensive abdominal pattern of *acutilabella* females. The abdominal pattern of *belladunni* of both sexes will distinguish them from all members of the dunni subgroup (Fig. 1).

Males of *belladunni* and *acutilabella* can be distinguished by the following characters as based upon *belladunni*.

Labellum. No acute process at tip as in *acutilabella*.

Palpi. With 5–6 long bristles on antero-lateral margin, the subapical bristle the longest, the others of about equal length. There are 5–6 bristles on the antero-lateral margin in *acutilabella*, but the first 3 are longest and stoutest, with the apical one the longest of the three, the remaining 2–3 bristles gradually becoming shorter posteriorly.

Abdomen. Tergites 2, 3, and 4 with narrow posterior dark bands interrupted dorsally; 5th and 6th solid black except on lateral margin (Fig. 1); not known to be polymorphic. The pattern in *acutilabella* is sometimes very similar to that of *belladunni* in Jamaica, but also sometimes with dark lateral extensions connecting 3rd and 4th segments at angle of tergite and sometimes with light paramedian extensions on 2nd and 3rd tergites.

Genitalia. Forceps with 6–7 primary teeth and 7–8 secondary teeth; lower tip of anal plate with 3–4 medium length bristles. *D. acutilabella* with about 6 primary and 9–10 secondary teeth on forceps, the lower tip of anal plate with subapical extension bearing one exceptionally stout long curved bristle.

Chromosomes. Metaphase plate preparations show 2 pairs of V's, one smaller than the other, and 2 pairs of rods of which one pair stains darker than the other. The lighter staining rod is the X-chromosome; the darker staining rod is the dot (4th) chromosome with added heterochromatin. Salivary chromosomes show 5 arms and a dot. This description is based upon stock No. H356.3. *D. acutilabella* has 2 pairs of V's, one pair of rods, and one pair of dots.

Distribution and Types. *Drosophila belladunni* belongs to the cardini species group of the subgenus *Drosophila*. It is known only from Jamaica, B. W. I., where it is usually collected in association with *acutilabella*. In the summer of 1958 *belladunni* made up 11% of 196 males of the two species collected at three localities in the lowlands along the north coast. At Hardware Gap (4,380 feet), however, *belladunni* made up 98% of the 56 males collected. From this data *belladunni* is considered an older member of the Jamaican fauna than *acutilabella*.

Holotype male and paratype males and females, from stock No. H356.3d from Hardware Gap, Jamaica, British West Indies, deposited in the University of Texas collection.

DISTRIBUTION

The distribution of the species of the cardini group in the West Indies is not fully known, but enough collecting has been done to emphasize several points (Table 1). *Drosophila acutilabella* is definitely absent on Puerto Rico; it is limited to southern Florida and the three islands indicated. Members of the dunni subgroup inhabit the Lesser Antilles and Puerto Rico, including the Virgin

TABLE 1

Distribution of the cardini group species in the Caribbean region

	Southern Florida	Cuba	Jamaica	Haiti	Puerto Rico	Lesser Antilles	Trinidad and South America
cardini	X	X	X	X	X	X†	X
acutilabella	X	X	X	X	..	..	..
belladunni	..	..	X	..	..	..	..
dunni subgroup	..	..	..	..	X	X‡	..
polymorpha	..	..	..	..	..	X§	X
Other spp.*	..	..	..	..	..	..	X

* *cardinoides*, *neomorpha* and undescribed sp.

† Grenada, St. Vincent, St. Lucia and Martinique only.

‡ All 8 islands visited.

§ Grenada only.

Islands; they definitely do not extend to Trinidad or to the mainland of South America.

The closest relatives of the dunni subgroup are *acutilabella* and *belladunni*, described above. They are sibling species, living sympatrically in Jamaica, and with the females being indistinguishable. However, *belladunni* is more frequent in the highlands while *acutilabella* is more frequent in the lowlands.

Hispaniola has been sampled only once and it is not known whether a member of the subgroup inhabits this large island. Similarly, collections in Cuba have been too fragmentary to be significant.

Drosophila polymorpha is the only cardini group species known to bridge the 87-mile gap between Trinidad and Grenada, aside from the widely distributed *cardini*, but it has not yet dispersed any farther north. It is interesting that this species is not polymorphic in Trinidad or Grenada as it is in Brazil (da Cunha 1949), the light form being the only one ever collected in the islands. Here is more evidence that marginal populations cannot afford the luxury of polymorphism.

POPULATION SAMPLES

The dunni forms are well established on all the islands collected, judging from the fact they are usually among the first three most common forms collected over natural or artificial bait. Tables 2 and 3 list a few examples of the frequencies of the most numerous species from two general habitats on the islands. The wood-

TABLE 2

The three dominant species from woodland habitat samples

	PR Jan. 31	SK Jan. 21	SL Jan. 17	BA Jan. 2	SV July 24
No. Species	7	9	9	5	7
No. Individuals	351	515	579	322	1,206
Percent willistoni group	56.7	59.6	54.2	93.5	14.6
Percent dunni subgroup	22.2	25.2	23.8	5.3	66.7
Percent others	17.9*	7.0‡	7.9‡	0.6‡	17.6†

* *simulans* (very few *melanogaster*); † *melanogaster*; ‡ *sturtevantii*.

TABLE 3

The three dominant species from domestic and semi-domestic habitat samples

	PR Aug. 18	PR Feb. 4	GU Aug. 3	MA July 31	SL Jan. 18	BA Jan. 5	GR July 22
No. species	9	14	15	13	10	9	13
No. individuals	890	394	690	665	1,448	793	831
Percent willistoni group	68.5	42.9	13.6	11.0	12.6	15.6	15.2
Percent dunni subgroup	6.0	(4.9)	(7.8)	13.4	75.0	49.7	(6.3)
Percent <i>melanogaster</i>	18.5	...	42.0	64.4	...	...	30.4
Percent <i>ananassae</i>	...	10.2	...	...	...	22.1	33.0
Percent others	...	22.1*	9.3†	...	3.8‡	...	...

* *mediodiffusa*; † *ornatipennis*; ‡ *metzii*.

land habitat includes such areas as the Caribbean National Forest in Puerto Rico, a government reserve forest in St. Vincent, and Turner's Hall Wood in Barbados. The domestic and semidomestic habitats include local secondary woods, cacao and banana fincas, orchards and garbage dumps. The dunni forms are more consistently common in the relatively undisturbed woodlands and they may be considered a characteristic part of the native fauna of the islands.

The sample from Barbados in Table 2 from an orchard is a selected sample in favor of the dunni forms. They were very numerous on a pile of freshly cut citrus branches and twigs. The sample from St. Lucia however, from a baited local secondary woods, is not selected. It is possible that the dunni forms are presently being transported from island to island by man and by other means, although not at high enough rate in most cases to obviate either the phenotypic identity of most island types or the genetic sterility found in some of the crosses.

HYBRIDIZATION TESTS

Two sets of mass matings were made independently by both investigators (Tables 4 and 5). Table 4 gives higher total numbers because the larvae were treated with yeasted kleenex. Table 5 includes a Puerto Rico strain and no control (homogamic) matings were made. A strain from St. Lucia was later tested by one set of mass matings (approx. 20 males and 20 females) with four other island strains. The tests were run for only one month and were not run to extinction as in the above crosses. The results are listed separately in Table 6. Twenty-five sets of 10 pair matings each were made and the results are listed in Table 7.

The first generation hybrids from all the original crosses except those involving the St. Lucia strain were inbred. The tests that gave an F_2 generation or more are listed in Table 8. Backcross tests were made with most of the hybrid females to usually both types of original strain males when the hybrid stock would not inbreed. The backcross offspring were allowed to inbreed for several weeks. After this time the number of vials that showed larvae out of the total number of vials for each set was recorded (Table 7). The original hybrid males were not tested for fertility in backcrosses.

From the tables it can be seen that all degrees of genetic relationship between

TABLE 4

Total number of hybrids and controls from two mass matings of approximately 20 ♀ and 20 ♂ each with yeasting method. Results of the second mass mating are shown in parentheses

♀	♂ ST	GU	BA	SV	GR
	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂
ST	498:362 (892:809)	64:46 (23:31)	273:205 (168:117)	7:2 (0:2)	0:2 (1:1)
GU	39:18 (29:32)†	567:391 (917:740)	587:559 (856:782)	79:64 (26:25)	20:26 (62:58)
BA	47:19 (39:15)†	937:215* (1,548:329)*	746:741 (1,501:1,278)	151:183 (13:61)	9:41 (10:101)
SV	87:52† (no count)	405:48* (232:36)*	642:602 (835:609)	730:675 (696:601)	711:659 (578:570)
GR	42:21† (10:13)†	828:5* (768:10)*	798:666 (852:592)	541:444 (944:825)	628:511 (706:638)

* One-half of males with abnormal abdomens.

† Approximately one-sixth of males with abnormal abdomens.

TABLE 5

Total number of hybrids from two mass matings of 20 ♀ and 20 ♂ each without yeasting method. Results of the second mass mating are shown in parentheses.

♀	♂ PR	ST	GU	BA	SV	GR
	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂
PR		31:12 (81:67)	8:14 (18:17)	15:18 (50:46)	1:1 (2:0)	0:0 (0:0)
ST	230:152 (109:54)		46:64 (74:45)	142:153 (111:104)	9:19 (1:3)	0:0 (4:1)
GU	6:1 (17:10)	53:10 (14:8)		141:129 (264:145)	56:44 (11:5)	55:44 (38:37)
BA	7:5 (4:10)	5:1 (15:8)	362:113 (137:18)		45:83 (30:67)	77:141 (35:51)
SV	1:0 (0:0)	8:6 (7:7)	34:3 (119:2)	13:30 (150:124)		20:29 (38:45)
GR	1:0 (5:3)	13:12 (17:13)	45:0 (90:2)	20:29 (107:101)	40:22 (16:6)	

TABLE 6

SL crosses for one month only

♀ ♂	♀	♂	♀ ♂	♀	♂
SL × SL	94	74	ST × SL	0	0
SL × ST	6	0	GU × SL	75	48
SL × GU	50	12	SV × SL	0	0
SL × SV	48	10	BA × SL	105	99
SL × BA	259	261			

the islands are exhibited from complete fertility in the F_2 as in the case of $PR \times ST$ (and reciprocal) and $SV \times GR$ (and reciprocal), to strong sexual isolation especially in the cases of PR and ST with SV and GR and with some of the tests with SL . A few dissections were made to check for sexual isolation; however,

TABLE 7
Pair matings in the dumni subgroup

♀	♂	No. vials fertile from 10 pairs	Total No.		χ^2_1 for a 1:1	P value	Average per fertile ♀	
			♀♀	♂♂			♀♀	♂♂
ST × ST		6	334	288	3.40	>.05	55.7	48.0
GU × GU		7	378	314	5.92	>.01	54.0	44.9
SV × SV		8	473	374	11.58	<.01	59.1	46.8
GR × GR		9	920	856	2.30	>.05	102.1	95.1
BA × BA		9	142	151	.276	>.50	15.8	16.8
ST × GU		1	4	3		..	4.0	3.0
ST × SV		0	...	...		..	...	...
ST × GR		0	...	...		..	...	...
ST × BA		6	39	42	not significant		6.5	7.0
GU × ST		2	17	7		..	8.5	3.5
GU × SV		4	45	41	not significant		11.3	10.3
GU × GR		3	40	36	not significant		13.3	12.0
GU × BA		10	423	381	2.2	>.05	42.3	38.1
SV × ST		1	1	2		..	1.0	2.0
SV × GU		9	412	20	significant		45.8	2.2
SV × GR		7	331	315	not significant		47.3	45.0
SV × BA		6	282	224	6.64	=.01	47.0	37.3
GR × ST		2	3	0		..	...	...
GR × GU		7	358	3	significant		51.1	0.43
GR × SV		7	594	530	3.64	>.05	84.9	75.7
GR × BA		6	256	210	4.54	>.01	42.7	35.0
BA × ST		1	1	0		..	...	...
BA × GU		10	370	164	significant		37.0	16.4
BA × SV		4	16	26	2.5	>.05	4.0	6.5
BA × GR		2	5	29	significant		2.5	14.5

TABLE 8
Total number of inbred hybrid offspring from crosses in Tables 4, 5 and 7

♀	♂	F_2	F_3	F_6
		♀♀ ♂♂	♀♀ ♂♂	♀♀ ♂♂
PR × ST		Fertile	Fertile	Fertile
ST × PR		Fertile	Fertile	Fertile
SV × GR		Fertile	Fertile	Fertile
GR × SV		Fertile	Fertile	Fertile
SV × GU		222:88	29:29	275:218*
BA × SV		217:144	None	
BA × GU		9:6	None	
GU × BA		3:2	None	
GU × BA		32:24†	None	

* Sample count from hybrid stock.

† Original parents one mated pair.

TABLE 9

Backcrosses of 23 ♀ ♀ per cross except where marked

Backcross	No. of Offspring		Inbred Offspring		Backcross	No. of Offspring		Inbred Offspring	
			No. of Fertile Vials					No. of Fertile Vials	
	♀/♂ × ♂	♀	♂	No. of Vials		♀/♂ × ♂	♀	♂	No. of Vials
GU/ST×ST	0	0	...		GU/SV×SV	111	95	0/5	
ST/GU×ST	0	0	...		BA/SV×SV	310	196	1/6	
*ST/SV×ST	90	53	0/2		†GR/ST×GR	10	10	1/1	
SV/ST×ST	221	82	1/6		GR/GU×GR	207	92	0/6	
†GR/ST×ST	173	71	1/5		‡GU/GR×GR	106	62	0/3	
ST/BA×ST	86	44	0/4		GR/BA×GR	235	174	1/7	
GU/ST×GU	0	0	...		ST/BA×BA	80	21	0/2	
ST/GU×GU	0	0	...		GU/BA×BA	194	185	3/5	
GU/SV×GU	202	80	2/3		BA/SV×BA	298	242	2/6	
GR/GU×GU	229	55	1/2		GR/BA×BA	432	259	3/6	
GU/BA×GU	233	118	1/4		§BA/GR×BA	255	207	2/6	
SV/ST×SV	89	46	1/4						

* 7 ♂♂
 † 12 ♂♂
 ‡ 18 ♂♂
 § 9 ♀♀

most of the low number hybrids are inferred to be due to sexual isolation. The two extremes in genetic relationship agree well with the geography of the islands (notice that St. Vincent and Grenada are "bridged" by the Grenadines).

In regard to sexual activity another inference may be made. BA males have a strong sexual drive, for they consistently give a high number of hybrids with other island females and produced the highest number of offspring in homogamic mass matings. The high sexual activity may be of a density-dependent nature since BA homogamic pair matings produced the lowest number of offspring compared to the other four homogamic matings (Table 7).

Some very obvious unequal sex ratios may be seen in the F_1 hybrids (Tables 4, 5, 6 and 7). The most consistent abnormal ratios are from the mating of GU males with other island females and from the mating of BA females with other island males. Tables 10 and 11 show this relationship. The data are extracted from the tables mentioned above. The reciprocal crosses of these matings usually produced a normal sex ratio.

Concerning the crosses with GU males, Table 10 shows a decrease in hybrid males with more or less increased distance from the island of Guadeloupe. The cline in sex ratio is illustrated in relation to the geography of the islands in Table 12. The data in the right hand column are taken from the backcross tests (Table 9). The reciprocal crosses, hybrid GU females to males of other islands, gave a 1:1 ratio with BA and SV males and a 2:1 ratio with GR males.

There are several factors to be considered here: the cline in phenotype, the sex ratio cline and the geographic position of the islands. Barbados is the most isolated island (92 miles from St. Lucia) and contains the darkest individuals. However it does not have as severe an abnormal sex ratio as the St. Vincent and Grenada strains. The St. Vincent and Grenada strains are phenotypically iden-

TABLE 10

Sex ratio cline of Guadeloupe males with females of other islands from five sets of crosses

♀ ♂	Cross	Females	Males	Ratio
GU × GU		567	391	1.4:1*
		917	740	1.2:1*
		378	314	1.2:1†
	Total	1,862	1,445	1.3:1*
BA × GU		937	215	4.4:1
		1,548	329	4.7:1
		370	164	2.3:1†
		362	113	3.2:1
		137	18	7.6:1
Total	3,354	839	4.0:1	
SL × GU		50	12	4.2:1
SV × GU		405	48	8.4:1
		232	36	6.4:1
		412	20	20.6:1†
		34	3	11.3:1
		119	2	58.5:1
Total	1,202	109	11.0:1	
GR × GU		828	5	165.6:1
		768	10	76.8:1
		358	3	118.9:1†
		45	0	
		90	2	45 :1
Total	2,089	20	104.5:1	

* Deviation from 1:1 ratio highly significant in homogamic matings.

† Totals from pair matings.

tical and produce a highly fertile F_2 ; however, crosses of the strains to GU males show a significant difference in sex ratio.

The nature of the lethality in the hybrid males has not yet been determined. Egg counts were not made, but it is assumed that the cross-lethal effect acts between the zygote and pupal stages since the control crosses of GU × GU gave a much higher proportion of males. That is, any male meiotic disturbance such as that leading to the "sex-ratio" effect analyzed by Sturtevant and Dobzhansky (1936) in *D. pseudoobscura* is ruled out. Also there is no reason to believe that a cytoplasmic factor is causing unequal sex ratios as reported by Malogolowkin (1958) for *D. willistoni* and *pauistorum*. Our unequal ratios are dependent on the genotype of the male, at least, and probably also of the female.

The backcrosses of the hybrid females to GU males show that even though the progeny have predominantly GU chromosomes the sex ratio cline is still in evidence. This gives some indication that the cross-lethal effect is an unfavorable interaction between a factor (or factors) on the other island X-chromosomes with the GU Y-chromosome.

The only cross involving GU males that gave sufficient numbers in the F_2 for comparison with the above data is that of SV x GU (Table 8). The hybrid mating produced a ratio of 222 females : 88 males or 28.4 percent males. This is the same proportion of males produced in the backcross of GU/SV X GU. Thus the cross lethal effect is acting in a consistent fashion regardless of the proportion of GU autosomes in the hybrids.

The F_3 generation gave a normal sex ratio indicating that the cross lethal effect had been nullified due either to more recombination of the original SV X chromosome by crossing over or due to selection for the GU X chromosome or most of it.

TABLE 11

Sex ratio cline of Barbados females with males of other islands from five sets of crosses

Cross ♀ ♂	Females	Males	Ratio
BA × ST	47	19	2.5:1
	39	15	2.6:1
	1	0	...†
	5	1	5:1
	15	8	1.9:1
Total	107	43	2.5:1
BA × GU	937	215	4.4:1
	1,548	329	4.7:1
	370	164	2.3:1†
	362	113	3.2:1
	137	18	7.6:1
Total	3,354	839	4.0:1
BA × SL	105	99	1.1:1‡
BA × BA	746	741	1.0:1
	1,501	1,278	1.2:1*
	142	151	1:1.1†
Total	2,389	2,170	1.1:1*
BA × SV	133	167	1:1.3‡
	13	61	1:4.7
	16	26	1:1.6‡
	45	83	1:1.8
	30	67	1:2.2
Total	237	404	1:1.7
BA × GR	9	41	1:4.6
	10	101	1:10.0
	5	29	1:5.8†
	77	141	1:1.8
	35	51	1:1.5‡
Total	136	363	1:2.7

* Deviation from 1:1 ratio highly significant in homogamic matings.

† Totals from pair matings.

‡ Deviation from 1:1 ratio not significant in heterogamic matings.

TABLE 12

Sex ratios obtained in crosses with Guadeloupe (GU) males

♀	♂	Degrees N. latitude	Miles through connecting islands	Percent ♂♂ from all parental crosses	Percent ♂♂ from hybrid ♀♀ × GU ♂♂
GU × GU		16° 15"	0	43.7	...
SL × GU		13° 55"	140	19.4	...
BA × GU		13° 10"	255	20.0	33.6
SV × GU		13° 15"	190	8.3	28.4
GR × GU		12° 5"	280	0.9	19.4

A sample count from the F₆ hybrid stock gave 44.2 percent males, which is more similar to the proportion of males regularly produced within the GU strain (43.7%) than within the SV strain (47.2%). There was also strong selection toward the GU phenotype after the second generation. The F₆ hybrid progeny could not be distinguished from the GU phenotype. Contamination of this stock by GU individuals is ruled out since biochemical studies on enzyme activity now in progress show that the stock is of hybrid origin.

Table 13 shows the sex ratios from each pair mating involving GU males. Three of the seven homogamic pairs produced significantly more females than

TABLE 13

Pair matings of Guadeloupe males with females of other islands

Pairs	GU × GU		BA × GU		SV × GU		GR × GU	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1	47	50	20	3	20	0	52	0
2	58	37*	30	8	119	0	50	0
3	35	46	34	24	106	5	144	3
4	68	44*	4	0	18	3	42	0
5	68	35*	114	51	14	8	54	0
6	72	60	28	3	4	0	4	0
7	30	42	57	18	65	1	12	0
8	..	..	26	4	16	2	..	..
9	..	..	40	43	50	1	..	..
10	..	..	17	10	..	..	..	..
Total	378	314	370	164	412	20	358	3

* Deviation from 1:1 ratio significant in homogamic matings.

males. This would not be emphasized if it were not for the fact that the two mass homogamic matings of the GU strain also produced significantly more females than males. The tendency toward abnormal ratios is thus already present in the GU strain and the effect is increased with crosses to the other island females. The GU females mated with other island males produced mostly normal sex ratios (except with ST males) in mass matings and with each pair mating. Therefore it does not appear as though the GU X-chromosomes carry any lethal or semi-lethal factors.

A tentative conclusion from the data at hand is that the cross-lethal effect

involving GU males is due to one or several factors on the other island X-chromosomes interacting abnormally with the GU Y-chromosome.

Matings with BA females to males of the other islands (Table 11) show an interesting sex ratio cline in that more females than males are produced with islands far to the north of Barbados; an equal number are produced with SL males (slightly north of Barbados), and more males than females are produced at the same latitude (St. Vincent) and south of Barbados (Grenada) (Table 14).

TABLE 14

Proportion of male offspring in crosses involving females from Barbados

♀ ♂	Degrees N. latitude	Miles through connecting islands	Percent ♂♂ from all parental crosses
BA × ST	18° 20"	555	28.7
BA × GU	16° 15"	255	20.0
BA × SL	13° 55"	92	48.5
BA × BA	13° 10"	0	47.6
BA × SV	13° 15"	100	63.0
BA × GR	12° 5"	180	72.7

The strains from Barbados and St. Lucia have given equal effects in sex ratio among themselves and with GU males. In this respect BA has closer affinities to SL than either SV or GR. Again it is seen that SV and GR differ from one another, this time in respect to BA females. The difference is highly significant using the totals from Table 11 in a 2 x 2 contingency table. The nature of the reverse in sex ratio is not yet known.

Backcrosses of hybrid BA females to males of BA and to males of other islands (Table 9) show that the cline, including the reversal in sex ratio, is nullified when the chromosomes are predominantly of either BA or the other parental type. In the former case most of the crosses approximate a 1:1 ratio. In the latter case most of the crosses approximate a 2:1 ratio. Too few hybrids were produced in pair matings with BA females to show the result for each pair.

CYTOLOGY

The number of strains used in the cytological analysis is more inclusive than is reported for the hybridization tests. Only the matings that produced new or otherwise unpredictable information on the ability of two strains to cross will be reported in this section along with the cytological results.

Figures 2 and 3 show the somatic metaphase chromosomes. They may be grouped into five distinct haploid types.

- Type 1. Two V's, one smaller than the other, one long rod with a proximal satellite (X chromosome) and a dot chromosome. Common to: SK, GU, MA, SL, BA, SV, and GR.
- Type 2. Two V's, one smaller than the other, one long rod with proximal satellite (X chromosome) and a small rod. Found only in Puerto Rico.



FIG. 2. Camera lucida drawings of the neurocyte chromosomes of the *dunni* subgroup (a-l) and of *belladunni* (m-n). Island strains are as follows: Puerto Rico (a-c), St. Thomas (d, ♀; e, ♂), Guadeloupe (f), St. Kitts (g), Martinique (h), St. Lucia (i), St. Vincent (j), Grenada (k), Barbados (l); Jamaica *belladunni* (m, early metaphase; n, late metaphase).

- Type 3. One large V, one J (autosome), one J (X chromosome) and a small rod. The Y is a dark staining heterochromatic rod. Found only in St. Thomas.
- Type 4. Two V's, one smaller than the other, and two rods, one stains darker than the other. The lighter staining rod is the X chromosome. Found only in *D. belladunni* from Jamaica.
- Type 5. Two V's, one rod (X chromosome) and a dot. Common to *D. acutilabella* from Florida, Cuba, Jamaica and Haiti. The configuration agrees with Stalker's description of *acutilabella* from St. Petersburg, Fla. (1953).

The salivary chromosomes of all the strains listed above were examined. They showed five arms and a dot radiating from the chromocenter. One of the arms is lightly staining in good preparations and bears a large puff at the distal end. Comparison of male and female salivaries suggests that this is the X chromosome. The dot, which is partly incorporated in the chromocenter, represents the fourth

chromosome. It is the same size and carries the same number of bands in all cases indicating that its size difference in metaphase configurations is due to shifts in heterochromatic material.

The other four strands represent the arms of the second and third metacentric chromosomes. All arms are distinguishable from one another by the characteristic appearance of the chromatin bands.

At least eight larval salivary gland preparations were examined from the original stocks. No inversions were found in the intrastain preparations except in the stock from Martinique which was heterozygous for inversion 2RB in the right arm of the second chromosome.

At least eight larvae, when they were available, were examined from most of the inter-island crosses. Five different inversions were found, one on each long arm; they have been designated as Xe, 2LB, 2RB, 3LB complex, and 3RB. For discussion, the strains used in these crosses may be divided into three groups: (1) *D. acutilabella* from Jamaica, Cuba, Haiti, St. Petersburg and Floral City, Florida; (2) *D. belladunni* from Jamaica; (3) the dunni subgroup from the Lesser Antilles and Puerto Rico. The inversion relations between them are shown diagrammatically in Fig. 8.

Initially, *D. belladunni* would not cross with any of the other two groups. However, further testing has shown that *belladunni* females will cross with males of BA, GR, GU, PR, MA and ST, the order indicating the relative ease

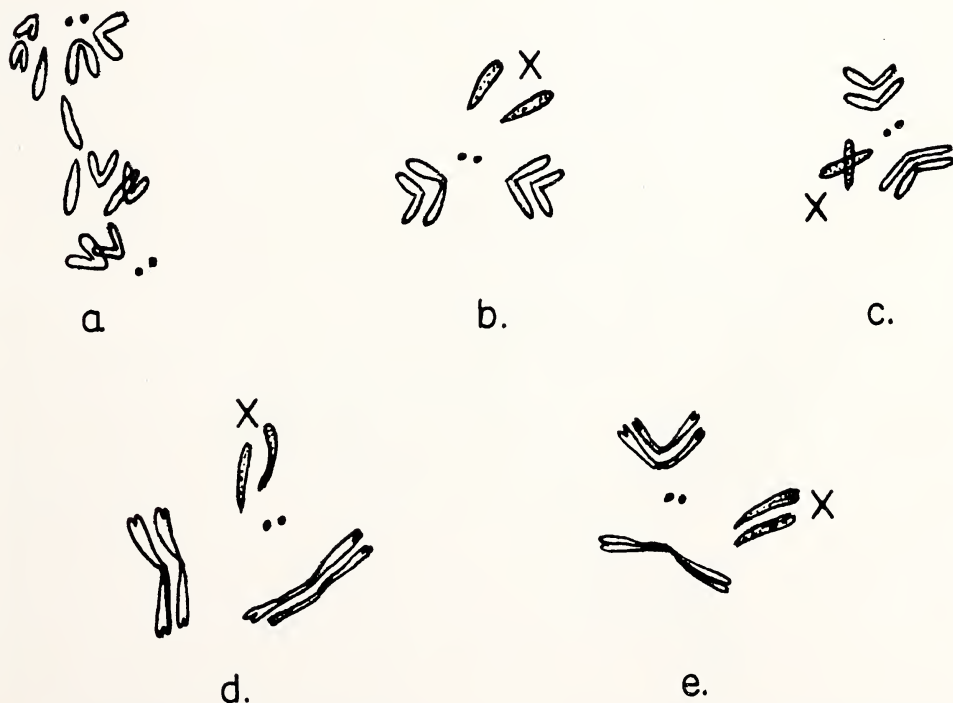


FIG. 3. Camera lucida drawings of the neurocyte chromosomes of the strains of *Drosophila acutilabella*; Jamaica (a, anaphase), St. Petersburg, Fla. (b), Floral City, Fla. (c), Cuba (d), Haiti (e).

with which the crosses can be made. The inversions have not yet been worked out.

The various strains of *acutilabella* crossed well among themselves and only one inversion was found; whenever the Haiti strain was one of the parents the progeny showed a complex inversion (3LB complex) in the left arm of the third chromosome (Fig. 5).

The five *acutilabella* strains would cross only with the Guadeloupe and St. Vincent strains of the *dunni* subgroup although they were tested with all of them (Table 15). The hybrid larvae examined for inversion differences were with the Jamaican strain of *acutilabella* crossed to Guadeloupe and St. Vincent. In both cases an inversion in the X-chromosome was found, Xe (Fig. 6). In addi-

TABLE 15

Hybrids obtained from crosses of *D. acutilabella* from Jamaica (J), Hispaniola (H), Cuba (C), St. Petersburg, Fla. (F) and Floral City, Fla. (Fl); *D. belladunni* from Jamaica (Jb); and two strains of the *dunni* subgroup, Guadeloupe (GU) and St. Vincent (SV).

♂ ♀	GU ♀ ♂	SV ♀ ♂	Jb ♀ ♂	J ♀ ♂	H ♀ ♂	C ♀ ♂	F ♀ ♂	Fl ♀ ♂
GU	..	..	0	6:0	1:0	2:0	3:0	1:0
SV	..	..	0	2:0	0	0	0	3:0
Jb	0*	0	143:99	0	0	0	0	0
J	2:1	7:1	0	..	..	..	..	..
H	1:0	2:0	0	..	..	..	..	..
C	2:1	5:0	0	..	..	..	..	..
F	0	3:0	0	..	..	..	..	..
Fl	0	2:0	0	..	..	..	..	..

* Some larvae were produced in a recent re-test; these are now being studied.



FIG. 4. Camera lucida drawing of the proximal inversion 3RB present in the progeny of *dunni* (Puerto Rico) × *dunni* (Guadeloupe).

tion, an inversion in the second chromosome, 2LB, was present in the Jamaica-Guadeloupe hybrids (Fig. 7). These are interspecific crosses and pairing was poor.

Crosses within the dunni subgroup showed three inversions, two of which have already been mentioned. The inversion 2RB, heterozygous in Martinique, appeared in some of the hybrid larvae in every cross of Martinique to other islands. The inversion 2LB, previously mentioned in the *acutilabella*-Guadeloupe cross, appears in all hybrid larvae of PR, GU, MA, SL, and BA crossed to SV and GR. The third inversion within the dunni subgroup appears in all hybrid larvae of GU, MA, SL, BA and SV crossed to PR and ST. This inversion is 3RB (Fig. 4).

Table 16 lists all the inversions actually observed except the complex inversion 3LB, homozygous in the Haiti strain of *acutilabella*. The degree of pairing of homologous arms is also indicated.

The inversion differences of the inter-island crosses (Fig. 8) divide the dunni subgroup into three distinct units: a northern unit (PR and ST), a middle unit (GU, MA, SL and BA) and a southern unit (SV and GR). This agrees with the genetic tests in that PR and ST are interfertile and SV and GR are interfertile. The fertility of the GU $\times$ SV hybrids is an exception to this and shows an unexpected relationship between the two islands. Further confirmation of this relationship is the fact that the strains from these two islands are the only ones in the Lesser Antilles that give at least a few hybrids with the five strains of *acutilabella*. Figure 8 illustrates the inter-island relationships by the inversions found in the hybrids.

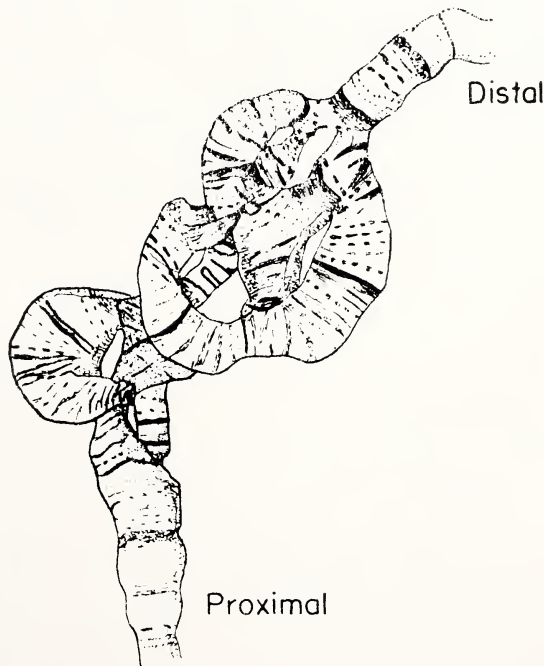


FIG. 5. Camera lucida drawing of the complex inversion 3LB present in the progeny of *acutilabella* (Haiti) $\times$ *acutilabella* (Jamaica).



FIG. 6. Camera lucida drawing of the salivary chromosomes of a female hybrid from the cross *dunni* (St. Vincent) $\times$ *acutilabella* (Jamaica). The inversion Xe is shown and the numerous regions of poor pairing (arrows).

DISCUSSION AND SUMMARY

A glance at Table 9 showing the results of the backcrosses in the *dunni* subgroup indicates that in the majority of the cases introgressive hybridization between island populations is theoretically possible. Direct fertile hybridization is possible between the two widely separated islands of St. Vincent and Guadeloupe, as well as the adjacent islands of Puerto Rico and St. Thomas, and of Grenada and St. Vincent.

However the genetic differences between the island populations are very noticeable. Most of the inter-island F_1 hybrids show a variable phenotype indicating the original types are heterozygous for factors controlling the abdominal pattern. This heterozygosity does not seem to depend on inter-island migration since each of the three main inversion groups (PR, ST; GU, MA, SV, BA; SV, GR) are homozygous for their own inversions. As far as our samples are ade-

quate (each island stock is derived from many females) this indicates a distinct isolation at least between the three main groups.

There is of course the possibility that selection acting on the segregates from the hybrids is favoring the inversion already established on the island that is receiving the migrants. The F_6 hybrids of SV $\times$ GU are phenotypically very constant and similar to the Guadeloupe parent. The fixation to one parental type in later hybrid generations (here only inbreeding and not backcrossing was allowed) supports Stebbins' (1959) recent statement that in animals selection after hybridization would be expected to act upon a specific adaptive type rather than any variety of types (hybrid swarm). Identification of the hybrid chromosomes is now in progress.

The increase in severity of abnormal sex ratios with distance in two series of inter-island crosses shows that differently integrated gene systems are being formed characteristic of each island population. Most impressive is the fact that St. Vincent and Grenada, although phenotypically identical, behave significantly different in crosses to males of Guadeloupe in the one case and to females of Barbados in the other. For other well documented cases of cross-lethals and semi-lethals see Crow (1942) and Patterson and Griffen (1944).

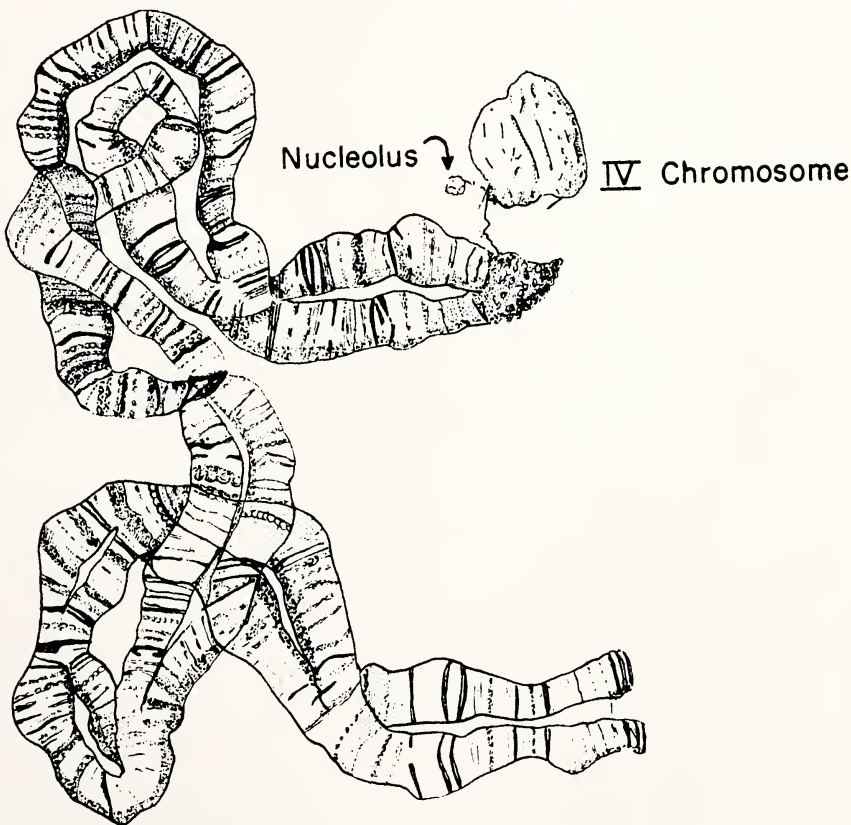


FIG. 7. Camera lucida drawing of the left arm of the II chromosome with the basal inversion 2LB present in the progeny of *dunni* (Guadeloupe) $\times$ *acutilabella* (Jamaica).

TABLE 16

Inversion differences and degree of pairing in the salivary chromosomes found in hybrids of inter-island crosses in the dunni subgroup and with *D. acutilabella* from Jamaica (J)

	PR	ST	GU	MA	SL	BA	SV	GR
PR		none good	3RB good	3RB 2RB-some poor	3RB poor	3RB poor	3RB 2LB poor	
ST			3RB good	3RB 2RB-some poor		3RB good		
GU				2RB-some good	none good	none good	2LB good	
MA								
SL				2RB-few good		none good		2LB good
BA				2RB good			2LB good	
SV				2LB 2RB-few good				none good
GR							none good	
J			Xe 2LB poor				Xe poor	

The cytological picture in the dunni subgroup is rather conservative in comparison to the genetic differentiation. Martinique has the only population carrying an inversion in the heterozygous condition. This inversion is restricted to Martinique. The other two inversions, 3RB and 2LB, appear only in hybrids in crosses of Puerto Rico and St. Thomas to all other islands, and St. Vincent and Grenada to all other islands.

The most noticeable characteristic of the dunni subgroup is the cline in abdominal pattern. Several types of clines have been reported in *Drosophila*. They have ranged from the temperature viability clines in *D. funebris* (Timofeeff-Ressovsky, 1933) through morphological gradients in *D. robusta* (Stalker and Carson, 1947) to clines in inversion frequencies in *D. pseudoobscura* (Dobzhansky, 1944), *D. robusta* (Carson and Stalker, 1947) and others. It is generally agreed that clines are the result of adaptation of local populations either directly or indirectly to environmental differences throughout the range of the organism. For *Drosophila* and many other organisms these environmental differences are not usually obvious because we must think and measure in terms of the microenvironment of the organism. This is a field that deserves intense investigation.

In the tropical lowlands the majority of *Drosophila* species are yellow. In the

tropical highlands there is a much higher frequency of dark species. Very few *Drosophila* species in the tropics have a continuous range from sea level to even five or six thousand feet (Heed, 1957b). Darker insects at higher altitudes are thought to be more protected from ultra violet radiations (Kalmus, 1941a). Also it has been demonstrated that darker *Drosophila* are more resistant to desiccation (Kalmus, 1941b). Even with this knowledge it would be mere speculation to assign a reason for the tremendous increase in black pigment in the dunni subgroup in the more southern parts of its range.

An alternative explanation for the cline in the dunni subgroup is the possibility that it is due to chance. This does not seem to be the case because of the regularity of the cline. The genetic make-up of the original founder population on each island was a chance phenomenon but there must be at present a constant and step-wise different selection pressure acting on each island population. For an excellent theoretical discussion of founder populations on peripheral islands see Mayr (1954).

There are three types of clines exhibited in the cardini group in the West Indies. The first two clines, abdominal pattern and sex ratio, are restricted to the dunni subgroup. The third type, chromosomal, must include *D. belladunni* in Jamaica in order to establish this as a true cline. The three clines are independent of one another although the first two follow mostly the same path. The dunni subgroup forms a sequence from north to south of light to dark abdomens but this

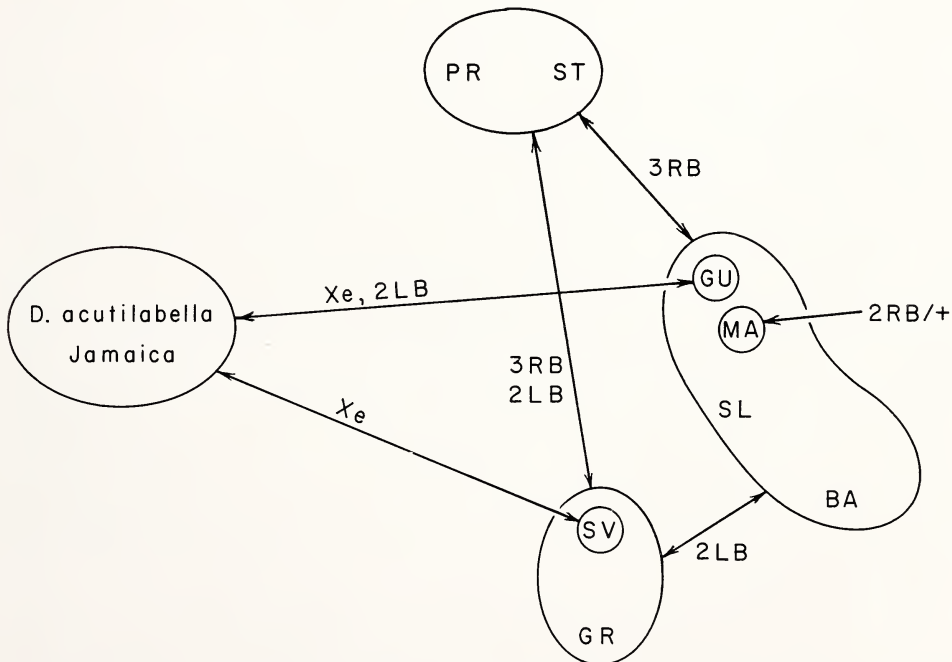


FIG. 8. Inversion differences among inter-island hybrids in the dunni subgroup and *D. acutilabella*. The Martinique strain is heterozygous for inversion 2RB. The island strains are: Grenada (GR), St. Vincent (SV), Barbados (BA), St. Lucia (SL), Martinique (MA), Guadeloupe (GU), St. Thomas (ST), Puerto Rico (PR).

TABLE 17

Summary of major *Drosophila* collecting localities in the West Indies

Collecting dates	Island	General localities
Dec. 31-Jan. 5, 1956	Barbados	Turner's Hall Wood, Blower's Wood, Grants Castle.
July 29, 1957	Barbados	Monkey Hill.
Jan. 13-19, 1956	St. Lucia	Castries, Barre de l'île, Pigeon Island.
Jan. 20-23, 1956	St. Kitts	Basseterre, Mt. Misery, Monkey Hill.
July 24-28, 1957	St. Vincent	Kingstown, Blue Lagoon, Soufriere Mountains.
July 20-23, 1957	Grenada	St. George, Grand Etang.
July 31-Aug. 1, 1957	Martinique	Fort de France, Morne Rouge.
Aug. 3-8, 1957	Guadeloupe	Matouba, Volcan Soufriere.
Aug. 11-13, 1957	St. Thomas	Signal Hill.
Jan. 30-Feb. 7, 1956	Puerto Rico	Carib. Natl. Forest at El Yunque and Sabana, USDA Exper. Sta. at Rio Piedras.
Aug. 16-19, 1957	Puerto Rico	USDA Exper. Sta. at Mayaguez.
Oct. 11-Nov. 1, 1957	Puerto Rico	Mayaguez, Ensenada, Cedra, Maricao, El Yunque.
Feb. 11-18, 1956	Haiti	Kenscoff, Furcy, Petionville.
Feb. 21-29, 1956	Jamaica	Bath, Hermitage Reservoir.
July 1-4, 1957	Jamaica	Montego Bay, Windsor.
Nov. 5-14, 1957	Jamaica	Kingston, Hardware Gap, Mona, Bath.
July 19-27, 1958	Jamaica	Windsor, Ocho Rios, Mt. Diablo, Hardware Gap, Hermitage Reservoir.

is not a perfect sequence. Barbados has the darkest form but is not the most southern island. Martinique is north of St. Lucia but it has the darker type.

The sex ratio cline with Guadeloupe males has the sequence: GU>SL>BA>SV>GR. This puts the Barbados form in the peculiar position of being the more closely related to the form from St. Lucia than the darker types from St. Vincent and Grenada. The inversion story also bears this out, since GU, SL and BA hybrids are all homozygous. Barbados was probably colonized from St. Lucia.

The chromosomal cline involves the addition (or subtraction) of heterochromatin on the dot chromosome. The metaphase of all island types south of St. Thomas show the usual dot chromosome. The Puerto Rico and St. Thomas forms have this chromosome enlarged and *D. belladunni* in Jamaica owns a heterochromatic rod in place of the dot. *D. belladunni* has definite affinities with the dunni subgroup (see species description) and thus the cline is probably valid in the sense that *belladunni* did not acquire the extra heterochromatin totally independently (or that the Puerto Rico and St. Thomas forms represent a *reduction* in heterochromatin).

The three clines represent genetic divergence in close relation to the geographic position of the islands. According to Mayr (1942) the presence of clines indicates continuities between adjacent populations. He was talking mainly about mainland populations, however. Our data show discontinuities and divergences yet they are still in a regular sequence or cline. The introgressive possi-

bilities and the clines in the dunni subgroup probably indicate very recent habitation of Puerto Rico and the Lesser Antilles instead of present day continuities. Barbados gives us a lower time limit for the establishment of its very dark population. Beard (1949) dates Barbados' recent uplift as post-Pleistocene. The habitation of Barbados was probably much more recent.

If the colonization of the Lesser Antilles by the dunni subgroup is indeed very recent then the affinities of its members are with the Greater Antilles rather than South America since the closest relatives are *D. acutilabella* and *D. belladunni*.

D. acutilabella should be taken as the standard salivary chromosome type in the cardini group since it crosses more readily with all other members of the group. Stalker (1953) reports that *acutilabella* from Florida will give sterile hybrids with *cardini*, *polymorpha* and *parthenogenetica* and fertile hybrids with *cardinoides*. The present work shows that *acutilabella* from its entire range will give sterile hybrids with the dunni subgroup strains from St. Vincent and Guadeloupe. If *acutilabella* is taken as the standard (Figure 8) there is no other choice than to follow the inversion steps from Jamaica to Grenada-St. Vincent and proceed north to Puerto Rico. The logical conclusion to this migration pattern would be to terminate again in Jamaica with *D. belladunni*.

However, there are two reasons for believing that this hypothesis may not be correct. The migration from Jamaica to the southern islands of the Lesser Antilles seems to be far more improbable than through Hispaniola to Puerto Rico and south. Also we believe that *belladunni* is the older form on Jamaica (see species description), inhabiting that island before *acutilabella*. The establishment of the dunni subgroup from the Greater Antilles at this point appears more likely than from South America but the dispersal route by which the Lesser Antilles were colonized is in doubt. For a discussion of migration patterns and chance dispersal of *D. willistoni* in the West Indies see Dobzhansky (1957).

The relation between *acutilabella* and *belladunni* is complex. They are sympatric in Jamaica and should be treated as sibling species since it is usually impossible to separate females in the field. Both species have affinities with the dunni subgroup. The arrangement of palpal bristles, an excellent distinguishing character in the cardini group, and the male genitalia of *belladunni* are similar to the dunni subgroup. Tests now in progress indicate that female *belladunni* will produce at least a few hybrids with males of most of the members of the dunni subgroup. *D. acutilabella* is not as morphologically similar to the subgroup but has produced sterile hybrids with the members from St. Vincent and Guadeloupe.

D. acutilabella may also be considered a sibling species of *D. cardini* in Florida. From the notes of a collecting trip with Dr. Marshall R. Wheeler in 1953, we agreed that *acutilabella* and *cardini* females could not be safely distinguished (see also Stalker, 1953). There is at the same time an obvious difference between *cardini* and *belladunni*. It seems as though *acutilabella* is quite plastic, "imitating" *cardini* in Florida and *belladunni* in Jamaica. The fact that *acutilabella* is also polymorphic adds to the confusion but does not invalidate the above observations. *D. cardini* is also found in Jamaica but is rarer than the other two species. This relationship deserves further investigation.

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A Nomenclatural Study of the Genus *Drosophila*

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About 1934 Dr. Patterson began studying the local species of *Drosophila* with a view to finding additional species which might serve as experimental animals in studies of comparative genetics. By 1938 it was apparent that there were many more species in the Austin area than had been supposed, and that many of them were undescribed. With the aid of Prof. A. H. Sturtevant the species were studied taxonomically as well as genetically, and a new phase of genetics research at the University of Texas began.

In those early days various graduate students assisted Dr. Patterson in the field collecting, for example Dean Parker, John Carpenter, Gordon Mainland, Robert Wagner, William Baker and myself. The taxonomic results of these collections were summarized in 1942 in the University of Texas Publication 4213 in which 46 new species were described. A resurgence of interest in *Drosophila* systematics followed this publication, as is shown from the data presented in Figure 1—a resurgence which has not yet lost its momentum. It is clearly evident that Dr. Patterson's influence in this field has been tremendous, and I feel that it is a distinct privilege to contribute this article to a volume honoring the man who was not only responsible to a considerable degree for the healthy development of modern *Drosophila* systematics, but who was also largely responsible for my own interest in it.

THE GENUS *DROSOPHILA*

The famous dipterist, C. F. Fallén, established the genus *Drosophila* in 1823 for *Musca funebris* Fabricius and the following eleven new Swedish species: *curvipennis*, *variegata*, *fenestrarum*, *transversa*, *obscura*, *tristis*, *fuscula*, *cinerella*, *flava*, *gramium*, and *glabra*. In addition to *funebris*, which was designated as the type species by Zetterstedt in 1847,* only five of Fallén's species are still considered as belonging to *Drosophila*: *fenestrarum*, *transversa*, *obscura*, *tristis*, and *flava*.

In the years immediately following Fallén's description of the genus a few new species were added sporadically but many of them were, in turn, relegated to other genera. In later years, however, and in spite of the narrowing concept of the generic limits, the growth of the genus has been remarkable. Figure 1 illustrates the number of species described in the genus by decades since 1823, as well as the number still considered to be valid members of the genus (the difference between the numbers in each sector). Relatively few species have been placed in *Drosophila* after being described in other genera; these species have not been tabulated.

* The earlier designation of *Drosophila cellaris* as the type species, by Curtis in 1833 and by Westwood in 1840, was invalid since *cellaris* was not an originally included species.

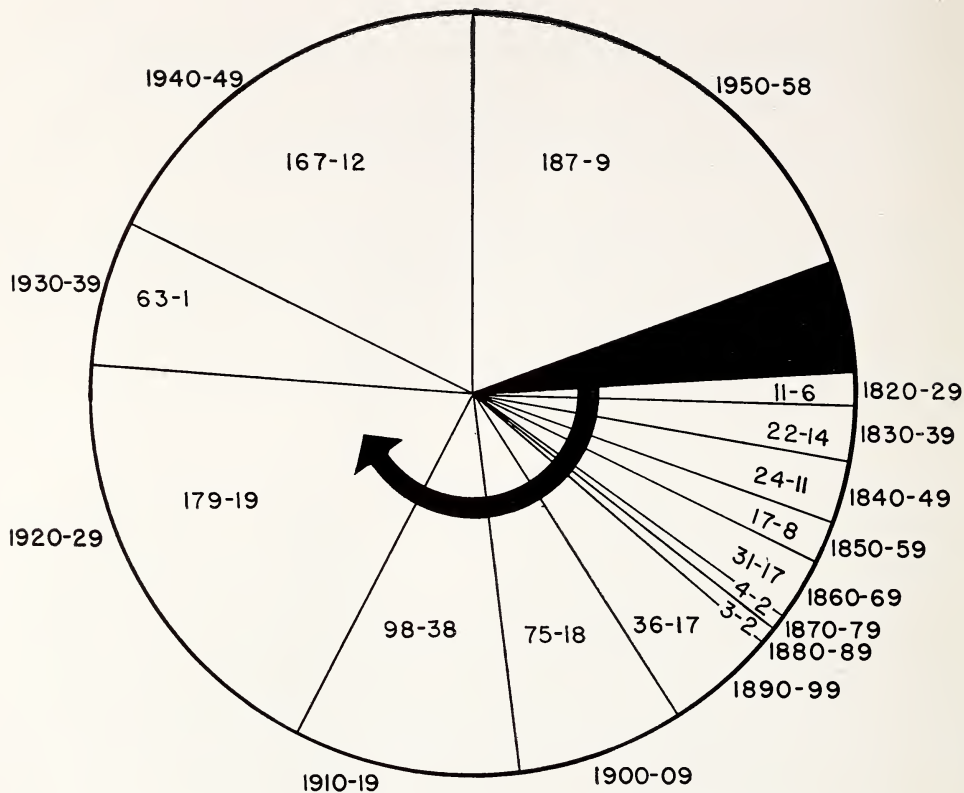


FIG. 1. Diagram illustrating the number of species described in *Drosophila* by decades since 1823. Numbers within the sectors represent the number of described species less the number no longer considered valid members of the genus by reason of generic transfer or synonymy.

At the time of writing, 920 species have been described in the genus *Drosophila*, about 85% of them since 1900. The spectacular peak of 179 species named between 1920 and 1929 represents, primarily, the efforts of three men, O. Duda, J. R. Malloch, and A. H. Sturtevant. Nearly 40% of the known species have been described since 1940, thus reflecting the renewed interest of many workers in many lands who are engaged in analyzing the multitude of species which inhabit the earth.

New Names Proposed for Junior Homonyms

In assembling the catalogue list which follows, instances of both primary and secondary homonymy have been found. The Rules of Nomenclature require that junior primary homonyms are to be corrected whenever discovered while junior secondary homonyms need substitute names only if a true biological homonymy is considered to exist. I am therefore proposing new names for all junior primary homonyms in the genus known to me, but action regarding most secondary homonyms is being postponed pending further biological information. Since names proposed for "varieties" will ultimately be ruled equivalent to subspecific names, nomenclaturally, then another series of primary homonyms will exist; these can be corrected when the need arises.

Drosophila (Hirtodrosophila) unicolorata* Wheeler, *nom. nov.

For *Hirtodrosophila unicolor* Malloch 1934 (Ins. Samoa, Pt. 6:293); = *Drosophila unicolor*, Patterson and Wheeler 1949, Harrison 1954.

Not *Drosophila unicolor* de Meijere 1914 (Tijd. Ent. 57:266).

The two species concerned are clearly referable to the genus *Drosophila*; I am therefore rejecting the junior name, *unicolor* Malloch, and proposing the substitute name, *unicolorata*. Malloch stated that although *Hirtodrosophila* was proposed as a subgenus of *Drosophila*, he felt that it was worthy of generic rank. Recent workers, however, consider it to be a valid subgenus.

Drosophila tendata* Wheeler, *nom. nov.

For *Drosophila dentata* Duda 1927 (Arch. Naturg. 91A12 (1925):145; 201).

Not *Drosophila longecrinita* var. *dentata* Duda 1924 (Arch. Naturg. 90A3:205; 242); = *Drosophila dentata*, Duda 1926 (Suppl. Ent. 14:65).

Duda (1924) described *dentata* as a variety of *longecrinita*, in the subgenus *Hirtodrosophila*, but elevated it to specific rank in 1926. He created a primary homonym by using the name *dentata* a second time for a wholly different species from South America.

Drosophila peruensis* Wheeler, *nom. nov.

For *Drosophila maculipennis* Duda 1927 (Arch. Naturg. 91A12 (1925):167).

Not *Drosophila maculipennis* Gimmerthal 1847 (Bull. Soc. imper. nat. Moscou 20:199); = *Diastata nebulosa* Fallén (Diastatidae).

Although *maculipennis* Gimmerthal has been removed to the Diastatidae, the name is preoccupied in *Drosophila*; since the species described by Duda came from Peru, I am using the name *peruensis* to replace his junior primary homonym.

Drosophila meijerei* Wheeler, *nom. nov.

For *Drosophila nigricolor* de Meijere 1911 (Tijd. Ent. 54:399), a new name proposed for *Drosophila nigra* de Meijere, not Grimshaw.

Not *Drosophila nigricolor* Strobl 1898 (1897; Mitt. Nat. f. Steiermark 34:266).

It is clear that de Meijere was not aware of the prior use of *nigricolor* when he proposed it as a substitute for his *nigra*, preoccupied by *nigra* Grimshaw. It seems appropriate to propose a new name honoring this eminent dipterist. Although the evidence is scanty, *Drosophila meijerei* probably belongs to the subgenus *Pholadoris*.

Drosophila neochracea* Wheeler, *nom. nov.

For *Drosophila ochracea* Duda 1927 (Arch. Naturg. 91A12 (1925):195).

Not *Drosophila ochracea* Grimshaw 1901 (Fauna Haw. 3:61).

A new name is needed to replace this junior primary homonym; to retain the general color significance of the name, the modification *neochracea* is proposed.

Drosophila adamsi* Wheeler, *nom. nov.

For *Drosophila quadrimaculata* Adams 1905 (Kans. Univ. Sci. Bull. 3:182).

Not *Drosophila quadrimaculata* Walker 1856 (Ins. Saund. 1, Dipt.:410); = *Leucophenga varia* (Walker) 1849 as *Drosophila varia* Walker.

Primary homonymy exists here in spite of the fact that Walker's species has been removed to another genus and is a synonym.

Leucophenga ornata Wheeler, *nom. nov.*

For *Drosophila ornatipennis* de Meijere 1914 (Tijd. Ent. 57:256);
= *Leucophenga ornatipennis*, Sturtevant 1921, Duda 1924.

Not *Drosophila ornatipennis* Williston 1896 (Trans. Ent. Soc. London, 1896, Pt. 3:407).

In naming his species de Meijere wrote "*Drosophila (Leucophenga) ornatipennis*", since he considered that breaking up *Drosophila* into many genera was poor taxonomy. He indicated by parentheses, however, those species which would belong in *Leucophenga* if it were recognized. As published, he created a junior primary homonym which requires a new name.

Microdrosophila zetterstedti Wheeler, *nom. nov.*

For *Drosophila nigriventris* Zetterstedt 1847 (Dipt. Scand. 6:2557);
= *Drosophila (Incisurifrons) nigriventris*, Duda 1934; = *Microdrosophila nigriventris*, Hackman 1954.

Not *Drosophila nigriventris* Macquart 1843 (1842. Mem. Soc. Sci. Arts Lille:416); = *Drosophila (Leucophenga) nigriventris*, de Meijere 1908;
= *Leucophenga nigriventris*, Sturtevant 1921, Duda 1924.

These two nominal species were originally described in the same genus, and Zetterstedt's species, being junior, requires a new name.

Drosophila multistriata Duda 1923 (Ann. Mus. Nat. Hung. 20:57).

Equals *Drosophila lineata* (de Meijere) 1911, as *Stegana lineata* (Tijd. Ent. 54:420).

Not *Drosophila lineata* van der Wulp 1886 (Dipt. Midd.-Sumatra Exped. iv(9):57).

Duda (1924) transferred *Stegana lineata* de Meijere to *Drosophila*, where it became a junior homonym of *lineata* van der Wulp. There is an available replacement name, however, since Duda has stated that his *multistriata* is the same as *lineata* de Meijere. If it should be shown later that this synonymy is in error, then de Meijere's species will require a new name.

Drosophila punctatonervosa Frey 1954 (Norw. Sci. Exped. Tristan da Cunha 26:32).

Equals *Drosophila poeciloptera* Duda 1940 (Ann. Mus. Nat. Hung. 33:26).

Not *Drosophila poeciloptera* (Duda) 1925, as *Paramycodrosophila poeciloptera* (Ann. Mus. Nat. Hung. 22:226), which equals *Drosophila poecila* Burla and Pavan 1953 (Rev. Brasil. Biol. 13:311).

Upon transferring *Paramycodrosophila poeciloptera* Duda 1925 to the genus *Drosophila*, and thus producing homonymy, Burla and Pavan (1953) unfortunately proposed a new name (*poecila*) for the senior homonym (*poeciloptera* Duda 1925) rather than the junior one (*poeciloptera* Duda 1940). *Drosophila poecila* is thus an absolute synonym of *poeciloptera* Duda 1925, and the junior name of 1940 still requires a substitute.

There is an available replacement name: *punctatonervosa* Frey 1954, which judging from the description, is a later name for the same species. If this synonymy, however, should later prove to be untrue, then *poeciloptera* Duda 1940 will require a new name.

Secondary Homonyms of Uncertain Validity

There are three instances of uncorrected secondary homonymy in the catalogue list which follows. In each case there is uncertainty regarding the biological reality of the homonymy. The pertinent facts are given below.

1. *Drosophila hypopygialis*. Duda described a *Tanygastrella hypopygialis* and later relegated *Tanygastrella* to the status of a subgenus of *Drosophila*. That specific name has also been used by Malloch in *Drosophila*. Until the position of *Tanygastrella* in *Drosophila* is verified, however, I am not willing to propose a new name for the junior homonym.

2. *Drosophila metallescens*. Malloch described a species with this specific name in *Spinulophila* which he considered to be a genus but which was originally proposed as a subgenus of *Drosophila*. Recent authors consider it to be approximately equivalent to the immigrans species group of the subgenus *Drosophila*. Malloch's species is, therefore, clearly a *Drosophila*, and the name is a junior homonym of *metallescens* de Meijere. I consider, however, that Malloch's species is a probable synonym of *monochaeta* Sturtevant; hence a new name is not required for it unless, and until, it should be shown to be a distinct species.

3. *Drosophila obscuricornis*. Duda transferred *Stegana obscuricornis* de Meijere into *Drosophila* where it becomes a junior homonym. Without a re-examination of the type of de Meijere's species, however, I believe that its position in *Drosophila* is to be questioned. If it is shown to be a true *Drosophila*, it should then be given a new name.

Annotated List of Species, Subspecies and Varieties

No one can deny that *Drosophila* is a very large genus. In the list which follows, about 750 presumably valid species are enumerated. In view of the fact that collecting has been relatively sparse in many areas of the world, it will not be surprising if the actual number of species reaches 1500. It will be even greater if the phenomenon of nearly identical sibling-species is as widespread as it now appears to be. It is for this reason, primarily, that this annotated list of species, subspecies and varietal names has been assembled. More than 1000 names have already been used in the genus, so that the creation of new names for the multitude of undescribed species is becoming a difficult task. To assist in the avoidance of homonymy the list has been arranged to show which names are unavailable for any other species or subspecies in the genus, and which names might be used again for some other species. Confusion can best be prevented, however, by avoiding the use of any of the names in the list for any future species or subspecies of *Drosophila*.

In preparing the list I have made extensive use of the earlier catalogue of Sturtevant (1921) and of the list of valid names published by Patterson and Wheeler (1949). I have attempted to bring these lists up to date and correct the

errors as far as possible. The manner of listing the five major categories of names is as follows:

1. *Species originally described in the genus Drosophila.* The name is in **bold-face** type followed by the author and date without intervening punctuation. These names may not be used again in the genus.

2. *Species transferred to the genus Drosophila.* The name is in **bold-face**, but with the author's name in parentheses, followed by the original generic reference. If the species is still considered to be a *Drosophila*, its name is not available for any other species. A number of species, having been transferred into *Drosophila*, were later removed from it again; only in special cases are they included in the list. Names of such temporary members of the genus are available for future use.

3. *Names of subspecies.* According to the Rules names of subspecies have equal standing with names of species as regards priority in homonymy. These names are in **bold-face**, with a statement regarding their status as subspecies. They may not be used again in the genus.

4. *Names originally proposed as "varieties."* There are 27 such names in the list; they are in *italics*, with a notation as to their varietal status. It seems probable that the new version of the Rules, which is to be published in the near future, will place varietal names on an equal status with those of subspecies, nomenclaturally; they will then be subject to the same rules governing homonymy as those now applicable to specific and subspecific names. It would seem prudent for *Drosophila* taxonomists to avoid the re-use of these varietal names for any new forms in the future.

5. *Names without status in nomenclature.* Also shown in *italics* are certain manuscript names, *nomina nuda*, invalid spellings, etc., which do not preoccupy for purposes of homonymy. There are several hundred such names in the literature; a few have been included in the list when, in the writer's opinion, they might have some bearing on nomenclatural problems.

The following abbreviations are employed: species (sp.), subspecies (subsp.), variety (var.), synonym (syn.), new name (n. n.), probable (prob.), possible (poss.), preoccupied, *i.e.*, a homonym (preocc.), considered (consid.), erroneous subsequent spelling (err. subseq. spelling), *nomen nudum* (nom. nud.), *species incerta* (sp. inc.), personal communication (pers. comm.).

ANNOTATED LIST OF NAMES IN THE GENUS DROSOPHILA

abbreviata de Meijere 1911:400. To *Leuco-phenga*.

aberrans Lamb 1914:334. Type of subg. *Dichaetophora*.

abregolineata Duda 1925:214.

abron Burla 1954a:170.

abure Burla 1954a:168.

acanthoptera Wheeler 1949b:171. Type of subg. *Sordophila*.

aceti Kollar 1851:205-6. Prob. syn. of *funebris* (Fabricius).

acuminata Collin 1952:199.

acuta Sturtevant 1927:370.

acutilabella Stalker 1953:345.

acutissima Okada 1956:139.

adamsi Wheeler 1959, *antea*. N. n. for *quadrimaculata* Adams, not Walker.

addisoni Pavan 1950:4.

adpersa Mik 1886:328. Syn. of *repleta* Wolaston.

adusta Loew 1862:231. To *Parascaptomyza*.

adyukru Burla 1954a:138.

afer Tan, Hsu, & Sheng 1949:200.

affinis Sturtevant 1916:334.

- agamse** Burla 1954a:129.
agbo Burla 1954a:104.
akabo Burla 1954a:109.
akai Burla 1954a:173.
akaju Burla 1954a:155.
alabamensis Sturtevant 1918b:38.
alafumosa Patterson & Mainland 1943:187.
alagitans Patterson & Mainland 1943:194.
albescens Frota-Pessoa 1954:281.
albicans Frota-Pessoa 1954:282. Poss. syn. of *albirostris* Sturtevant.
albiceps de Meijere 1914:258. To *Leuco-phenga*.
albicincta de Meijere 1908:156. To *Leuco-phenga*.
albicornis de Meijere 1915a:58. (the invalid original spelling, *abicornis*, was emended by de Meijere). To *Mycodrosophila*.
albifrontata Malloch 1934a:301.
albilabris Zetterstedt 1860:6425. (as first published, was attributed to "Roth, in litteris"). To *Amiota*.
albincisa de Meijere 1911:409.
albipes Walker 1852:410. Sp. inc.
albirostris Sturtevant 1921:78.
albofasciata Macquart 1851:277. To *Leuco-phenga*.
alboguttata Wahlberg 1838:22. To *Amiota*.
albolimbata Duda 1924a:216, & 1924b:256.
albomarginata Duda 1927:173.
albomicans Duda 1924a:209, & 1924b:245. Nom. nud. in Duda 1923:47. Syn. of *nasuta* Lamb.
albonotata de Meijere 1911:408.
allopunctata Becker 1900:64. To *Chymomyza*.
alboralis Momma & Takada 1954:98.
albostrigata Malloch 1924b:352.
albovittata Duda 1926a:83, 87. N. n. for *sulfurigaster* Duda, as an invalid replacement; syn of *nasuta* Lamb.
aldrichi Patterson & Crow 1940:251.
alexandrei Cordeiro 1951:1.
alfari Sturtevant 1921:75.
algonquin Sturtevant & Dobzhansky 1936:575.
alladian Burla 1954a:175.
alpina Burla 1948:274.
alternata de Meijere 1911:402.
alternolineata Duda 1925:213.
altiplanica Brncic & Santibañez 1957:69.
amabalis de Meijere 1911:405. To *Mycodrosophila*.
ambigua Pomini 1940:157.
americana Spencer 1938:169, as subsp. of *virilis*; now consid. sp.
amoena Loew 1862:230. To *Chymomyza*.
ampelophila Loew 1862:231. Syn. of *melanogaster* Meigen.
amplipennis Malloch 1934b:442.
analys Macquart 1843:415. Sp. inc.
ananassae Doleschall 1858:128.
ancep Patterson & Mainland 1944:39.
andalusiaca Strobl 1906:372.
andina Dobzhansky & Pavan 1943:59.
angularis Okada 1956:128.
angusta de Meijere 1915a:57.
angustibucca Duda 1925:218.
angustipennis de Meijere 1911:413. To *Diatatidae*.
annularis Sturtevant 1916:327. N. n. for *annulata* Williston, not Fallén.
annulata (Fallén 1813:250, in *Notiphila*). To *Drosophila* by Zetterstedt in 1847; later moved to *Periscelidae*.
annulata Williston 1896:409. Preocc.; n. n. is *annularis* Sturtevant.
annulimana Duda 1927:117.
annulipes Duda 1924a:209, 221, & 1924b:250. Nom. nud. in Duda 1923:58.
anomalipes Grimshaw 1901:62.
anuda Curran 1936:43.
anyi Burla 1954a:145.
apectinata Duda 1931:194.
apicata Thomson 1869:596. To *Scaptomyza*.
apicifera Adams 1905:185. To *Leucophenga*.
appendiculata Malloch 1934b:441. Type of subg. *Chusqueophila*.
approximata Zetterstedt 1847:2557. Syn. of *fasciata* Meigen (Duda 1935a).
aracea Heed & Wheeler 1957:36.
araicás Pavan & Nacur 1950:264.
arapuan da Cunha & I. Pavan 1947:36.
ararama Pavan & da Cunha 1947:28.
arassarí da Cunha & Frota-Pessoa 1947:32.
araucana Brncic 1957a:82.
araúna Pavan & Nacur 1950:268.
argentata de Meijere 1914:258. To *Leuco-phenga*.
argenteifrons Wheeler 1954:50.
argentina de Meijere 1924:46. To *Leuco-phenga*.
arizonensis Patterson & Wheeler 1942:96.
asozana Okada 1956:87.
astioidea Duda 1923:42.
aterríma Duda 1940:28, 48.
athabasca Sturtevant & Dobzhansky 1936:576.
atkinsoni (Miller 1921:302, in *Leucophenga*). Syn. of *funnebris* (Fabricius).
atra Walker 1852:412. Sp. inc.
atrata Burla & Pavan 1953:307.
atropyga Duda 1924a:215, as var. of *montium*.

- auraria** Peng 1937:23.
aurea Patterson & Mainland 1944:46.
aureata Wheeler 1957:83.
australica Duda 1923:59. Poss. syn. of *obsoleta* Malloch.
austrorepleta Dobzhansky & Pavan 1943:50.
 Syn. of *repleta* Wollaston.
austrosaltans Spassky 1957:57.
azteca Sturtevant & Dobzhansky 1936:577.
baeomyia Wheeler 1949a:145. Syn. of *latifasciaeformis* Duda.
balneorum Sturtevant 1927:369.
balteata Bergroth 1894:75. Syn. of *melanogaster* Meigen.
bandeirantorum Dobzhansky & Pavan 1943:30.
bangi Burla 1954a:143.
baole Burla 1954a:187.
basdeni Wheeler 1957:94.
baseogrisea Duda 1924a:221, & 1924b:257.
basilaris Adams 1905:184. To *Leucophenga*.
bellula Bergroth 1894:75. To *Leucophenga*.
bellula Williston 1896:410. Preocc.; n. n. is *pulchella* Sturtevant.
berryi Cockerell 1923:332. Fossil sp.
betari Dobzhansky & Pavan 1943:48.
biarmipes Malloch 1924c:64.
bicolor de Meijere 1911:399. To *Lissocephala*.
bicoloripes Malloch 1926:31. To *Chymomyza*.
bifasciata Pomini 1940:163. (*bifasciata* & *bilineata* were both original spellings; *bilineata* being preocc., *bifasciata* is an available replacement name).
bifilum Frota-Pessoa 1954:284.
bifurca Patterson & Wheeler 1942:85.
bilimbata Bezzi 1928:159. Syn. of *nasuta* Lamb.
bilineata Williston 1896:409. To *Zygothrica*.
bilineata Pomini 1940:155. Preocc.; replacement name is *bifasciata* Pomini.
bimaculata Loew 1865:183. To *Leucophenga*.
binotata de Meijere 1914:257.
biopaca Sturtevant 1942:37. Syn. of *sturtevanti* Duda.
bipectinata Duda 1923:52.
bipunctata Patterson & Mainland 1943:194.
biradiata Duda 1923:50.
bistriata de Meijere 1911:397. To *Zaprionus*, subg. *Phorticella*.
bizonata Kikkawa & Peng 1938:532.
bocainensis Pavan & da Cunha 1947:18.
bocainoides Carson 1954:150.
boletina Duda 1927:202.
boliviana Duda 1927:198.
boliviensis Duda 1927:215, as var. of *kertes-zina* Duda.
borealis Patterson 1952:20.
brachynephros Okada 1956:126.
brevicarinata Patterson & Wheeler 1942:88.
brevicornis Duda 1935a:76.
brevis Walker 1852:411. Sp. inc.
briegeri Pavan & Breuer 1954:459.
bromeliae Sturtevant 1921:72.
bromelioides Pavan & da Cunha 1947:7.
brouni Hutton 1901:91. Officially suppressed in favor of *immigrans* Sturtevant.
brunetti Chaudhuri & Mukherjee 1941:216.
brunnea de Meijere 1911:401.
brunneipalpa Dobzhansky & Pavan 1943:53.
brunneipennis Malloch 1923:617.
bryani Malloch 1934a:310.
busckii Coquillett 1901:18. (Invalid original spelling, *buskii*, was a typographical error). Type of subg. *Dorsilopha*.
buzzatii Patterson & Wheeler 1942:97.
calceolata Duda 1926a:94, 105.
californica Sturtevant 1923:9.
caliginosa Lamb 1914:341.
calloptera Schiner 1868:239.
camargoi Dobzhansky & Pavan 1950:6.
camaronensis Brncic 1957a:95.
cameraria Haliday 1833:174.
campestris Burla 1950b:9. Prob. syn. of *crocina* Patterson & Mainland.
canalineae Patterson & Mainland 1944:50.
canalinioides Wheeler 1957:92.
canapalpa Patterson & Mainland 1944:40.
cancellata Mather 1955:550.
canescens Duda 1927:210.
capnoptera Patterson & Mainland 1944:47.
caponei Pavan & da Cunha 1947:4.
capricorni Dobzhansky & Pavan 1943:14.
carbonaria Patterson & Wheeler 1942:103.
cardini Sturtevant 1916:336.
cardinoides Dobzhansky & Pavan 1943:21.
caribea Sturtevant 1916:335. Syn. of *ananas-sae* Doleschall.
carinata Grimshaw 1901:70.
carinata Duda 1923:41. Preocc.; n. n. is *latifrontata* Frota-Pessoa.
carsoni Wheeler 1957:95.
castanea Patterson & Mainland 1944:51.
caxiensis Cordeiro 1952:304.
cellaris (Linné 1758:597, in *Musca*). Sp. inc.; consid. *Drosophila*, especially *D. funebris*, by older authors; prob. to Phoridae (see Schiner 1864:278, and Duda 1935a:84).
centralis Patterson & Mainland 1944:57, as subsp. of *pallidipennis*.
chagrinsensis Stalker & Spencer 1939:111.
cheda Tan, Hsu & Sheng 1949:199.
chinoi Okada 1956:162.

- cilifemur** Villeneuve 1923:28. Syn. of *immi-grans* Sturtevant.
- cilitarsus** Hering 1940:293.
- cincta** de Meijere 1911:395. To *Leucophenga*.
- cinctifrons** de Meijere *i. litt.* of Duda 1924a:227. Invalid citation; sp. is *Chymomyza cinctifrons* de Meijere 1924:47.
- cinerea** Patterson & Wheeler 1942:71.
- cinerella** Fallén 1823:7. Becker 1926:43 lists "*Drosophila cinerella* Meigen 1830" as syn. of *Discocerina plumosa* (Fallén 1823) of the Ephydriidae, meaning *cinerella* Fallén as understood by Meigen; to *Scaptomyza* (Basden, pers. comm.).
- circumdata** Duda 1926a:82, 84. Prob. to *Chaetodrosophilella*.
- clarkii** Hutton 1901:91. Syn. of *funebrius* (Fabricius).
- clunierus** Duda 1923:51.
- coffeata** Williston 1896:409.
- coffeina** Schiner 1868:238.
- cognata** Grimshaw 1901:69.
- colocasiae** Duda 1924b:252.
- colorata** Walker 1849:1110.
- comoe** Burla 1954a:205.
- compressiceps** Duda 1923:55.
- compressifrons* of Hennig 1941:151. Err. subseq. spelling for *compressiceps*.
- confusa** Staeger 1844:16.
- congesta** Zetterstedt 1847:2558. To *Microdrosophila*.
- conspicua** Grimshaw 1901:59.
- converga** Heed & Wheeler 1957:22.
- convergens** de Meijere 1911:400. To *Orthostegana* (Hendel 1914); to *Stegana* (Sturtevant 1921); to *Oxyphortica* (Duda 1923).
- convexa** Malloch 1934a:303.
- coracina** Kikkawa & Peng 1938:523.
- cordata** Sturtevant 1942:34.
- costata** Zetterstedt 1838:776. To *Chymomyza*.
- crassa** Patterson & Mainland 1944:52.
- crassifemur** Grimshaw 1901:66.
- crocina** Patterson & Mainland 1944:34.
- crockeri** Curran 1936:44.
- crucigera** Grimshaw 1902:86.
- cubana** Townsend 1954:339, as subsp. of *tropicalis*.
- curvapex** Frota-Pessoa 1954:296.
- curvicapillata** Duda 1923:49.
- curviceps** Okada & Kurokawa 1957:8.
- curvinervis* Duda 1924a:204, as var. of *longecrinita* Duda.
- curvipennis** Fallén 1823:4. To *Protostegana*.
- cuzcoica** Duda 1927:120.
- daruma** Okada 1956:155.
- debilis** Walker 1849:1109. Sp. inc.
- decemguttata** Walker 1852:411. To *Diastatidae*.
- decemseriata** Hendel 1936:98.
- decipiens** Duda 1923:55.
- deflecta** Malloch 1924d:36.
- deflexa** Duda 1924a:222.
- deltaneuron** Bryan 1938:40.
- denieri** Blanchard 1938:362.
- dentata* Duda 1924a:205, as var. of *longecrinita*; raised to sp. by Duda 1926a:65, 69. (Nom. nud. in Duda 1923:42).
- dentata** Duda 1927:201. Preocc.; n. n. is *tentata* Wheeler.
- denticeps** Okada & Sasakawa 1956:26.
- diana** Burla 1954a:192.
- dibi** Burla 1954a:126.
- dilacerata** Becker 1919:208. Consid. *Scaptomyza* by Duda 1927, but descr. & wing fig. indicate *Drosophila*.
- dimidiata** Loew 1862:230. To *Mycodrosophila*.
- dispar** Mather 1955:570.
- distincta** Egger 1862:780. To *Chymomyza*.
- divisa** Duda 1927:187. (*diversa* is an err. subseq. spelling in Patterson & Wheeler 1949).
- dobzhanskii** Patterson 1943:82.
- dorsalis** Walker 1865:128.
- dorsata** Duda 1924a:207, 220, & 1924b:248. (Nom. nud. in Duda 1923:56).
- dorsivitta** Walker 1861:330. Sp. inc.
- dreyfusi** Dobzhansky & Pavan 1943:61.
- dubia** Sturtevant 1921:73. To *Clastopteromyia*.
- dudai** Malloch 1934b:444.
- dumuya** Burla 1954a:185.
- duncani** Sturtevant 1918a:446.
- dunni** Townsend & Wheeler 1955:61.
- dyula** Burla 1954a:178.
- dyaramankana** Burla 1954a:196.
- earlei** Sturtevant 1916:329.
- elliptica** Sturtevant 1942:35.
- elongata** Sturtevant 1927:372.
- emarginata** Sturtevant 1942:36.
- emulata** Chaudhuri & Mukherjee 1941:216.
- enderbii** Hutton 1902:174. To Ephydriidae.
- enigma** Malloch 1927:6.
- equinoxialis** Dobzhansky 1946:209.
- errans** Malloch 1933:21. N. n. for *similis* Lamb, not Williston; syn. of *ananassae* Doleschall.
- erythrophthalma** (Panzer 1794:24, in *Musca*). Sp. inc., poss. not *Drosophila* (Duda 1935a).
- euronotus** Patterson & Ward 1952:158.
- excepta** Malloch 1934a:308.
- excita** Giglio-Tos 1893:14. To Ephydriidae.
- exigua** Grimshaw 1901:72.
- ezoana** Takada & Okada 1958:134.

- facialba** Heed & Wheeler 1957:19.
facialis Adams 1905:183.
fasciata Meigen 1830:84. Consid. by some authors as prior name (page preference) for *melanogaster* Meigen.
fasciata Dufour 1839:49. Preocc., but consid. syn. of *testacea* von Roser by Duda 1935a.
fasciola Williston 1896:410.
fascioloides Dobzhansky & Pavan 1943:42.
fenestrarum Fallén 1823:6.
fenestrata de Meijere of Duda 1923:36. Invalid citation from museum label; valid name is *Zaprionus (Phorticella) fenestrata* (Duda) 1923.
ferruginea Becker 1919:209.
ficuspila Kikkawa & Peng 1938:531.
fima Burla 1954a:165.
finigutta Walker 1859:126. To *Lauxaniidae*.
finitima Lamb 1914:340.
flava Fallén 1823:7. Often consid. *Scaptomyza* but Collin (1953:150) consid. it *Drosophila*, *fenestrarum* species group.
flaveola (Meigen 1830:66, in *Notiphila*). To *Scaptomyza* acc. to many authors; to *Hydrellia* (Ephydriidae) by Duda 1934.
flaviceps Grimshaw 1901:63.
flavipennis Zetterstedt 1838:777. To *Scaptomyza*.
flavipes (Meigen 1830:108, in *Opomyza*). Consid. *Drosophila* by some older authors; Hennig (1937:31) makes it syn. of *Phyllosmyza securicornis* Fallén in *Milichiidae*.
flaviseta Adams 1905:184. To *Leucophenga*.
flavissima Duda 1929:419.
flavohalterata Duda 1925:198.
flavohirta Malloch 1924b:354.
flavolineata Duda 1927:157.
flavomontana Patterson 1952:21.
flavopilosa Frey 1919:14.
flavopinicola Wheeler 1954:47.
flavorepleta Patterson & Pavan 1952:114, as subsp. of *fulvimacula*.
flexa Loew 1865:182.
florae Sturtevant 1916:339.
floricola Sturtevant 1942:42. Type of subg. *Phloridosa*.
forcipata Collin 1952:198. Poss. syn. of *andalusiaca* Strobl.
formosana Duda 1926b:50, as var. of *immi-grans* Sturtevant (as "*tripunctata* Becker"); raised to sp. by Duda 1926a:83.
formosana Sturtevant 1927:368, as var. of *immigrans* Sturtevant. Preoc.; syn. of *formosana* Duda.
fraburu Burla 1954a:183.
fracticosta Lamb 1914:329. To *Mycodrosophila*.
fragilis Wheeler 1949b:191.
framire Burla 1954a:128.
frolovae Wheeler 1949b:175.
frontalis Williston 1896:413. To *Leucophenga*.
frontata de Meijere 1916:204. To *Microdrosophila*.
fronto Walker 1852:410. Sp. inc.
fruhstorferi Duda 1924a:211, 218, & 1924b:255.
fuliginea Patterson & Wheeler 1942:80. Prob. syn. of *californica* Sturtevant.
fulvalineata Patterson & Wheeler 1942:106.
fulvimacula Patterson & Mainland 1944:42.
fulvimaculoides Wasserman & Wilson 1957:137.
fumipennis Duda 1925:220.
fumosa Pavan & da Cunha 1947:14.
fundomaculata Duda 1925:209.
funebri (Fabricius 1787:345, in *Musca*). Type of gen. & subg. *Drosophila*.
fungicola Villeneuve 1921:158. Syn. of *unistriata* Strobl.
fusca Coquillett 1900:264. Sp. inc.
fuscimana Zetterstedt 1838:776. To *Chymomyza*.
fuscipennis Duda 1927:198.
fuscithorax Malloch 1924b:353.
fuscoamoeba Bryan 1934:438.
fuscohalterata Duda 1925:197.
fuscolineata Duda 1925:213.
fuscovittata Harrison 1954:106.
fuscula Fallén 1823:7. To *Diastatidae*.
gasci Brncic 1957a:92.
gaucha Jaeger & Salzano 1953:205.
gibberosa Patterson & Mainland 1943:195.
gibbinsi Aubertin 1937:169.
gibbosa de Meijere 1914:264. To *Leucophenga*.
gigantea Thomson 1869:596. Poss. *Leucophenga* (Kahl 1917:392); poss. *Curtonotum* (Malloch 1934b:437); true *Drosophila* (Sambrosky, pers. comm.).
gigas Duda 1925:216.
gilva Burla 1956:263.
glabra Fallén 1823:8. To *Camillidae*.
glabrifrons Duda 1925:196.
gracilipes Duda 1940:27, 39, as var. of *finitima* Lamb; prob. syn. of *latifasciaeformis* Duda (Burla 1954a).
gracilis (Duda 1924a:192, & 1924b:253, in *Tanygastrella*). To *Drosophila* by Duda 1926a:99, with *Tanygastrella* as subgen. of *Drosophila*.
graminum Fallén 1823:8. To *Scaptomyza*.

- grandis** Kikkawa & Peng 1938:543.
gratiosa de Meijere 1911:404. To *Mycodrosophila*.
grimshawi Oldenberg 1914:23. N. n. for *variiegata* Grimshaw, not Fallén.
grischuna Burla 1950a:620. Syn. of *confusa* Staeger.
grisea Patterson & Wheeler 1942:72.
griseicollis Becker 1919:209.
griseola Zetterstedt 1847:2562. To *Scaptomyza*.
griseolineata Duda 1927:161.
grossipalpis Lamb 1914:328. To *Leucophenga*.
guarajá King 1947:48.
guaramunú Dobzhansky & Pavan 1943:39.
guarani Dobzhansky & Pavan 1943:36.
guarú Dobzhansky & Pavan 1943:37.
guinensis Duda 1924a:209. Err. subseq. spelling for *novoguineensis* Duda.
guttifera Walker 1849:1110.
guttiventris de Meijere 1908:331. N. n. for *maculiventris* de Meijere, not van der Wulp. To *Leucophenga*.
guyénoti Burla 1948:277. Poss. syn. of *deflexa* Duda.
haleakalae Grimshaw 1901:64.
hamatofila Patterson & Wheeler 1942:91.
hawaiiensis Grimshaw 1901:60.
helvetica Burla 1948:276.
heterobristalis Tan, Hsu, & Sheng 1949:204.
hexastigma Patterson & Mainland 1944: 43.
hexastriata Tan, Hsu, & Sheng 1949:201.
hirsuta Duda 1926a:94, 97. Proposed as n. sp. or var., but cited, p. 97, as var. of *fenestrarum*.
hirticornis de Meijere 1914:261.
hirtipes Lamb 1914:337.
hirtiscutellata Sturtevant 1927:372.
histrio Meigen 1830:85.
histrioides Okada & Kurokawa 1957:4.
hoeckeri Brncic 1957a:76. Prob. syn. of *nigricruria* Patterson & Mainland.
hoozani Duda 1923:54.
huilliche Brncic 1957a:85.
humeralis Grimshaw 1901:64.
hyalipennis Duda 1927:119.
hydei Sturtevant 1921:101.
hydeoides Patterson & Wheeler 1942:84.
hypocausta Osten-Sacken 1882:245.
hypopygialis (Duda 1924a:192, & 1924b:254, in *Tanygastrella*). To *Drosophila* by Duda 1926a:96, with *Tanygastrella* as subg. of *Drosophila*.
hypopygialis Malloch 1934a:307.
icteroscuta Wheeler 1949b:184.
iki Bryan 1934:439, as var. of *nigra* Grimshaw.
illata Walker 1860:168. Sp. inc.
illota Williston 1896:415.
imeretensis Sokolov 1948:1007.
immatura Walker 1849:1108. Sp. inc.
immigrans Sturtevant 1921:83.
imparata Walker 1859:126. Syn. of *ananassae* Doleschall (de Meijere).
impudica Duda 1927:196.
inaequalis Grimshaw 1901:69.
inca Dobzhansky & Pavan 1943:44.
incana Meigen 1830:86. To *Scaptomyza*.
inconspicua de Meijere 1914:262.
infuscata Grimshaw 1901:63.
ingrata Haliday 1833:174. Syn. of *obscura* Fallén (Duda 1935a).
innocua (Malloch 1934a:294, in *Hirtodrosophila*).
innubila Spencer 1943:94.
inornata Malloch 1923:617.
insulana Schiner 1868:240. To *Leucophenga*.
insularis Dobzhansky 1957:41.
intermedia Duda 1927:125, 151, as var. of *adusta* in *Scaptomyza* which was consid. subg. of *Drosophila*. To *Parascaptomyza*.
interrupta Duda 1923:45.
inversa Walker 1861:331. To *Clastopteromyia*.
invicta (Walker 1857:130, in *Helomyza*). Consid. *Drosophila* by some older authors; to *Leucophenga* (Czerny); to *Trichiasiphenega* (Duda); to *Paraleucophenga* Hendel (Hennig 1941:150) whose type species, *trisetata* Hendel, is syn. of *invicta*.
iri Burla 1954a:180.
iroko Burla 1954a:171.
iroquois Sturtevant & Dobzhansky 1936:576, as subsp. of *affinis*.
itambacuriensis da Cunha 1955:119, as subsp. of *neocardini*.
jacobsoni Duda 1926a:65, as var. of *latifrons* Duda.
johni Pokorny 1896:63. To *Mycodrosophila*.
jordanensis Frota-Pessoa 1945:473.
jucunda Lamb 1914:339.
kallima Wheeler 1957:87.
kauluai Bryan 1934:439.
kertészina Duda 1925:222.
kikkawai Burla 1954b:47.
kirki Harrison 1959:303.
komaii Kikkawa & Peng 1938:525.
krugi Pavan & Breuer 1954:462.
kulango Burla 1954a:172.
kuntzei Duda 1924a:218.
kuoni Burla 1954a:190.
kuscheli Brncic 1957b:394.
kweichowensis Tan, Hsu, & Sheng 1949:205.

- lacertosa** Okada 1956: 158.
laticola Patterson 1944: 102.
lacteoguttata Portschinsky 1892: 226. To *Amiota*.
laeta Zetterstedt 1847: 2553, as var. of *transversa* Fallén.
lambi Duda 1940: 48. N. n. for *pallipes* Lamb, not Dufour.
lamellitarsis Duda 1936: 350.
lanaiensis Grimshaw 1901: 60.
lata Becker 1907: 54. To *Amiota*, subg. *Paraphortica*.
latebuccata Duda 1927: 203.
latecarinata Duda 1927: 170.
lateralis Walker 1860: 169.
latestriata Becker 1908: 157. Syn. of *unistriata* Strobl (Duda 1924c).
latifascia de Meijere 1914: 261. (*latifasciata* is err. subseq. spelling).
latifasciaeformis Duda 1940: 22.
latifrons Adams 1905: 182.
latifrons Duda 1926a: 64. Preocc.; invalidly proposed as n. sp. to include *carinata* & *astioidea* as vars.; n. n. is *latifrontata* Frota-Pessoa.
latifrontata Frota-Pessoa 1945: 480. N. n. for *latifrons* Duda, not Adams (and for *carinata* Duda, not Grimshaw; see Frota-Pessoa 1945, *op. cit.*). Type of subg. *Hirtodrosophila*.
lativittata Malloch 1923: 618.
lativittata Malloch 1924d: 36. Preocc.; n. n. is *palustris* Spencer.
lebanonensis Wheeler 1949a: 143. Prob. syn. of *victoria* Sturtevant.
leonis Patterson & Wheeler 1942: 82.
levigata Burla 1956: 261.
levis Mather 1955: 561. Syn. of *bryani* Malloch.
limbata von Roser 1840: 62.
limbata Williston 1896: 414. Preocc.; n. n. is *nebulosa* Sturtevant.
limbinervis Duda 1925: 215.
limbipennis de Meijere 1908: 156. To *Leucophenga*.
limbiventris Duda 1925: 212.
limensis Pavan & Patterson 1947: 26.
limpiensis Mainland 1941: 160, as subsp. of *macrospina*.
linearepleta Patterson & Wheeler 1942: 79.
linearis Walker 1852: 411. Sp. inc., prob. not *Drosophila*.
lineata van der Wulp 1886: 57.
lineata (de Meijere 1911: 420, in *Stegana*). Preocc.; replacement name is *multistriata* Duda (see remarks in text).
lineolata de Meijere 1914: 254.
litorella (Meigen 1838: 374, in *Hydrellia*). Sp. inc., prob. not *Drosophila* (Duda 1935a).
littoralis Meigen 1830: 87.
lividinervis Duda 1923: 53.
lividipennis Duda 1924a: 216. Err. subseq. spelling for *lividinervis* Duda.
longala Patterson & Wheeler 1942: 71.
longecrinita Duda 1924a: 204, & 1924b: 242. (Nom. nud. in Duda 1923: 42).
longicornis Patterson & Wheeler 1942: 90.
longifrons Duda 1923: 48.
longiseta Grimshaw 1901: 68.
longitarsis Duda 1931: 195.
lucida Segúy 1938: 351.
lugens Duda 1926a: 71, 76.
lugubrina Duda 1924a: 224. Syn. of *littoralis* Meigen.
lugubripennis Duda 1927: 199.
lulumahu Bryan 1938: 39.
lundstroemi Duda 1935a: 72.
lurida Walker 1860: 169.
lutea Kikkawa & Peng 1938: 533.
luteipes Sturtevant 1921: 74, as var. of *splendida*; to *Clastopteromyia*.
lutzii Sturtevant 1916: 340.
macropolia Patterson & Mainland 1944: 54.
macroptera Patterson & Wheeler 1942: 105.
macrospina Stalker & Spencer 1939: 110.
macularis Villeneuve 1921: 157. Syn. of *picta* Zetterstedt.
maculata Dufour 1839: 49. To *Leucophenga*.
maculifrons Duda 1927: 122.
maculinotata Okada 1956: 145.
maculipennis Gimmerthal 1847: 199. To *Diasatidae*.
maculipennis Duda 1927: 167. Preocc.; n. n. is *peruensis* Wheeler.
maculiventris van der Wulp 1897: 142. Consid. syn. of *repleta* Wollaston.
maculiventris de Meijere 1908: 155. Preocc.; n. n. is *guttiventris* de Meijere. To *Leucophenga*.
maculosa Coquillett 1895: 317, in Johnson, D. W., 1895. To *Leucophenga*.
maculosa Mather 1955: 560. Preocc.; n. n. is *novamaculosa* Mather.
magnarcus Frota-Pessoa 1951: 407.
magnabadia Patterson & Mainland 1943: 196.
magnafumosa Stalker & Spencer 1939: 112.
magnaquinaria Wheeler 1954: 48.
magnipectinata Okada 1956: 113.
mahican Sturtevant & Dobzhansky 1936: 576, as subsp. of *athabasca*.
mainlandi Patterson 1943: 147.
makinoi Okada 1956: 135.
mallochi Frota-Pessoa 1946: 155. N. n. for *lati-*

- vittata* Malloch 1924, not Malloch 1923; syn. of *palustris* Spencer.
- mangabeirai** Malogolowkin 1951:432.
- manonoensis** Harrison 1954:101.
- mansura** Adams 1905:185. To *Leucophenga*.
- maracaya** Wheeler 1957:88.
- marginata** Duda 1924a:209, & 1924b:244. (Nom. nud. in Duda 1923:46).
- marginella** Zetterstedt 1838:777. To *Diastatidae*.
- marjoryae** Harrison 1954:105.
- marmoria** Hutton 1901:91. Sp. inc., poss. is *hydei* Sturtevant.
- martensis** Wasserman & Wilson 1957:151.
- mauiensis** Grimshaw 1901:67.
- maura** de Meijere 1911:406. To *Pararhinoleucophenga*.
- mbettie** Burla 1954a:149.
- mediocris** Frota-Pessoa 1954:275.
- mediodelta** Heed & Wheeler 1957:21.
- mediodiffusa** Heed & Wheeler 1957:27.
- medioimpressa** Frota-Pessoa 1954:300.
- medionotata** Frota-Pessoa 1954:288.
- medioobscurata** Duda 1925:217.
- medioparva** Heed & Wheeler 1957:28.
- mediopicta** Frota-Pessoa 1954:290.
- mediopictoides** Heed & Wheeler 1957:24.
- mediopunctata** Dobzhansky & Pavan 1943:26.
- mediosignata** Dobzhansky & Pavan 1943:24.
- mediotriata** Duda 1925:223.
- mediovittata** Frota-Pessoa 1954:280.
- megaspis** Bezzi 1908b:191.
- meigeni* Duda 1935a:74, 90, as var. of *obscura*; consid. sp. by Basden 1954:617.
- meijerei** Wheeler 1959, *antea*, N. n. for *nigricolor* de Meijere, not Strobl.
- meitanensis** Tan, Hsu, & Sheng 1949:204.
- melanderi** Sturtevant 1916:337.
- melanica** Sturtevant 1916:332.
- melanissima** Sturtevant 1916:333.
- melanogaster** Meigen 1830:85. Type of subg. *Sophophora*.
- melanogaster** Macquart 1843:415. Preocc.; syn. of *fenestrarum* Fallén (Duda).
- melanopalpa** Patterson & Wheeler 1942:77.
- melanoptera** Duda 1927:158.
- melanosoma** Grimshaw 1901:68.
- melanospila** Walker 1859:126. To *Lauxanidae*.
- melanura** Miller 1944:86.
- mellea** Becker 1919:208.
- mercatorum** Patterson & Wheeler 1942:93.
- meridiana** Patterson & Wheeler 1942:99.
- mesophragmatica** Duda 1927:205.
- mesostigma** Frota-Pessoa 1954:269.
- metallescens** de Meijere 1914:265. To *Lissocephala*.
- metallescens** (Malloch 1934a:312, in *Spinulophila*). Prob. syn. of *monochaeta* Sturtevant.
- metallica** Sturtevant 1921:73. To *Clastopteromyia*.
- metzii** Sturtevant 1921:78.
- mexicana** Macquart 1843:416. Sp. inc.
- mexicana** (Wheeler 1949b:164, in *Paramyco-drosophila*). Preocc.; n. n. is *mexicoa* Wheeler.
- mexicoa** Wheeler 1954:54. N. n. for *mexicana* (Wheeler), not Macquart.
- microlabis** Segúy 1938:351.
- micromelanica** Patterson 1941:394.
- miki** Duda 1924a:213.
- minuta** Walker 1852:412. Sp. inc.
- minuta* Duda 1926a:66, 70, as var. of *dentata* Duda 1924.
- miranda** Dobzhansky 1935:377.
- mirim** Dobzhansky & Pavan 1943:62. Syn. of *latifasciaeformis* Duda.
- mitis** Curran 1936:43.
- modesta** Sturtevant 1916:338. Syn. of *tripunctata* Loew.
- mojavensis** Patterson & Crow 1940:251, as subsp. of *mulleri*; later consid. sp.
- mojú** Pavan 1950:19.
- mokonfim** Burla 1954a:131.
- molokaiensis** Grimshaw 1901:67.
- monochaeta** Sturtevant 1927:368.
- montana** Stone, Griffen, & Patterson 1941:172.
- monticola** Grimshaw 1901:69.
- montium** de Meijere 1916:205.
- morena** Frota-Pessoa 1954:283.
- moriwakii** Okada & Kurokawa 1957:9.
- moronu** Burla 1954a:134.
- morensis** da Cunha 1955:119, as subsp. of *neocardini*.
- mulleri** Sturtevant 1921:101.
- multipunctata** Loew 1866:50. Syn. of *guttifera* Walker.
- multispina** Okada 1956:143.
- multistriata** Duda 1923:57.
- munda** Spencer 1942:58.
- mutabilis** Adams 1905:187. To *Leucophenga*.
- mutandis** Tan, Hsu, & Sheng 1949:198.
- mycetophaga** Malloch 1924b:351.
- nana** Williston 1896:416. To *Clastopteromyia*.
- nannoptera** Wheeler 1949b:177.
- narinosa** Frota-Pessoa 1945:476. N. n. for *nasalis* Duda, not Grimshaw.
- narragansett** Sturtevant & Dobzhansky 1936:577.
- nasalis** Grimshaw 1901:66.

- nasalis** Duda 1925:194. Preocc.; n. n. is *narinosa* Frota-Pessoa.
- nasuta** Lamb 1914:346.
- nebulosa** Sturtevant 1916:327. N. n. for *limbata* Williston, not von Roser.
- neocardini** Streisinger 1946:111.
- neochracea** Wheeler 1949, *antea*. N. n. for *ochracea* Duda, not Grimshaw.
- neocordata** Magalhães 1956:275.
- neocelliptica** Pavan & Magalhães 1950:13.
- neoguaranunú** Frydenberg 1956:57.
- neomorpha** Heed & Wheeler 1957:33.
- neorepleta** Patterson & Wheeler 1942:78.
- neosaltans** Pavan & Magalhães 1950:16.
- neozelandica** Harrison 1959:290.
- nepalensis** Okada 1955:388.
- nicholsoni** Malloch 1927:4.
- nigerrima** Lamb 1914:331. To *Mycodrosophila*.
- nigra** Grimshaw 1901:62.
- nigra** de Meijere 1908:153. Preocc.; n. n. was *nigricolor* de Meijere, also preocc.; n. n. is *meijerei* Wheeler.
- nigra** Duda 1926a:65, 68, as var. of *latifrons* Duda.
- nigriceps** Meigen 1838:378. Consid. syn. of *obscura* Fallén.
- nigricincta** Frota-Pessoa 1954:289.
- nigricolor** Strobl 1898b:266.
- nigricolor** de Meijere 1911:399. N. n. for *nigra* de Meijere, not Grimshaw; preocc.; n. n. is *meijerei* Wheeler.
- nigricosta** Malloch 1926:30. To *Neotanygastrella*.
- nigricurria** Patterson & Mainland 1943:136.
- nigrifemur** Duda 1927:192.
- nigrifrons** Malloch 1934a:304.
- nigrimana** Meigen 1830:87. To *Chymomyza*.
- nigrita** Haliday of authors. Err. subseq. spelling for *ingrata* Haliday, possibly intended as an emendation.
- nigrithorax** Strobl 1893b:132, as var. of *fenestrarum*.
- nigriventris** Macquart 1843:416. To *Leucophenga*.
- nigriventris** Zetterstedt 1847:2557. Preocc.; to *Microdrosophila* with n. n. *Microdrosophila zetterstedti* Wheeler.
- nigrobrunnea** Lamb 1914:332. To *Mycodrosophila*.
- nigrodunni** Heed & Wheeler 1957:35.
- nigrofemorata** Duda 1926a:72, 96, 110.
- nigrohalterata** Duda 1925:195.
- nigrohylei** Patterson & Wheeler 1942:84.
- nigromaculata** Kikkawa & Peng 1938:537.
- nigromelanica** Patterson & Wheeler 1942:100.
- nigropunctata** van der Wulp 1892:216. Consid. syn. of *repleta* Wollaston.
- nigrosparsa** Strobl 1898b:267. Type of subg. *Spinodrosophila*.
- nigrospiracula** Patterson & Wheeler 1942:81.
- nigrovittata** Malloch 1924b:352. To *Dettopomyia*.
- nikananu** Burla 1954a:160.
- nipponica** Kikkawa & Peng 1938:531.
- nitens** Buzzati-Traverso 1943:2. Syn. of *rufifrons* Loew.
- nitidapex** Bigot 1891:279. Sp. inc., prob. not *Drosophila*.
- nitidithorax** Malloch 1927:5.
- nitidiventris** Macquart 1835:551. Consid. syn. of *fenestrarum* Fallén.
- niveopunctata** Dufour 1846b:318. Nom. nud. acc. to Oldenberg 1914:25 ff.
- nixifrons** Tan, Hsu, & Sheng 1949:202.
- nodosa** Duda 1926a:94, 103.
- nokogiri** Okada 1956:84.
- notabilis** Lamb 1914:329. Prob. to *Hypselythrea*.
- novamaculosa** Mather 1956:65. N. n. for *maculosa* Mather, not Coquillett.
- novamexicana** Patterson 1941a:535, as subsp. of *virilis*, later raised to sp.
- novemariata** Dobzhansky & Pavan 1943:55.
- novoguineensis** Duda 1923:46. Err. subseq. spellings are *novoguineensis* and *guineensis*.
- novopaca** Mather 1956:65. N. n. for *opaca* Mather, not Williston.
- nubiluna** Wheeler 1949b:188.
- nutrita** Duda 1935b:33.
- obesa** Loew 1872:102. To *Rhinoleucophenga*.
- obscura** Fallén 1823:6.
- obscurata** de Meijere 1911:410.
- obscuricolor** Duda 1927:190.
- obscuricornis** Grimshaw 1901:71.
- obscuricornis** (de Meijere 1915b:94 in *Stegana*). Consid. *Drosophila* by Duda 1924a & b.
- obscurifrons** Grimshaw 1901:72.
- obscuripennis** Loew 1865:183. To *Leucophenga*.
- obscurioides** Pomini 1940:149. Syn. of *obscura* Fallén.
- obsoleta** Malloch 1923:616. Consid. by Malloch (1925) as prob. same as "*australis* Duda," prob. meaning *australica* Duda 1923; if true, Malloch's name is prior (Dec. 14 vs. Dec. 24).
- occidentalis** Spencer 1942:60.
- ochracea** Grimshaw 1901:61.
- ochracea** Duda 1927:195. Preocc.; n. n. is *neochracea* Wheeler.

- ochracella** Hendel 1936:98. Prob. to *Zygothrica* (Burla 1956).
- ochrifrons** Duda 1924a:223, & 1924b:258.
- oenopota** (Scopoli 1763:337, in *Musca*). Sp. inc., prob. not *Drosophila* (Duda 1935a).
- ohioensis** Spencer 1940b:303, as subsp. of *macrospina*.
- olae** Grimshaw 1901:66.
- oldenbergi** Duda 1924a:204.
- omogoensis** Okada 1956:82.
- onca** Dobzhansky & Pavan 1943:40.
- onychophora** Duda 1927:208.
- opaca** Williston 1896:411. To *Clastopteromyia*.
- opaca** Mather 1955:558. Preocc.; n. n. is *novopaca* Mather.
- opisthomelaina** Nolte & Stoch of Nolte 1958:519. Manuscript name based on prior use in the unofficial *Drosophila* Information Service. Syn. of *yakuba* Burla.
- oralis** Duda 1923:44.
- orbitalis** Sturtevant 1916:336. To *Zygothrica*.
- orbospiracula** Patterson & Wheeler 1942:70.
- ordinaria** Coquillett 1904:190.
- orkui** Brncic & Santibañez 1957:69.
- ornatifrons** Duda 1927:162.
- ornatipennis** Williston 1896:407.
- ornatipennis** de Meijere 1914:256. Preocc.; to *Leucophenga*, with n. n. *Leucophenga ornata* Wheeler.
- osornina** Brncic 1957a:97.
- pachea** Patterson & Wheeler 1942:87.
- pallida** Zetterstedt 1847:2571. To *Parascapto-myza* (Basden 1957:209).
- pallida** Williston 1896:415. Preocc.; n. n. is *willistoni* Sturtevant.
- pallidipennis** Dobzhansky & Pavan 1943:32.
- pallipes** Dufour 1846a:323. Sp. inc., prob. to Diastatidae.
- pallipes** Lamb 1914:342. Preocc.; n. n. is *lambi* Duda.
- palpalis** Adams 1905:185. To *Leucophenga*.
- palustris** Spencer 1942:63. N. n. for *lativittata* Malloch 1924, not 1923.
- panamensis** Malloch 1926:28.
- para** Pavan & Burla 1950:22.
- parabocainensis** Carson 1954:149.
- paracanalinea** Wheeler 1957:93.
- parachrogaster** Patterson & Mainland 1943:197.
- paradoxa** Lamb 1918:159. To *Clastopteromyia*.
- paraguayensis** Duda 1927:185.
- paraguttata** Thompson 1957:98.
- paralevigata** Burla 1956:261.
- paramediotriata** Townsend & Wheeler 1955:62.
- paramelanica** Patterson 1942b:12, as subsp. of *melanica*; should be consid. sp.
- paranaensis** de Barros 1950:266.
- parapunctipennis** Duda 1924a:205. Nom. nud. in Duda 1923:44 as var. of *punctipennis*.
- pararepleta** Dobzhansky & Pavan 1943:52. Now consid. subsp. of *mercatorum*.
- parasaltans** Magalhães 1956:276.
- paravibrissina** Duda 1924a:218, & 1924b:248.
- parenti** Villeneuve 1921:157. Syn. of *littoralis* Meigen.
- parthenogenetica** Stalker 1953:347.
- parva** Grimshaw 1901:65.
- pattersoni** Pipkin 1956:251.
- paucilineata** Burla 1957:38.
- paucipunctata** Grimshaw 1901:62.
- paulista** Dobzhansky & Pavan 1943:10. Syn. of *willistoni* Sturtevant.
- paulistorum** Dobzhansky & Pavan 1949:301.
- pavani** Brncic 1957a:88.
- pectinipes** Duda 1927:177. Manuscript name, prob. not intended for use in nomenclature.
- pengi** Okada & Kurokawa 1957:11. Equals "*melanissima*" of Kikkawa & Peng 1938.
- peninsularis** Patterson & Wheeler 1942:92.
- perkinsi** Grimshaw 1901:59.
- persimilis** Dobzhansky & Epling 1944:7.
- peruensis** Wheeler 1959, *antez*, N. n. for *maculipennis* Duda, not Gimmerthal.
- peruviana** Duda 1927:204.
- phalerata** Meigen 1830:83.
- picta** Zetterstedt 1847:2567; (As first published, was credited to "Staeg. in litteris").
- picticornis** Grimshaw 1901:57.
- pictifrons** Duda 1927:182.
- pictipennis** Kertész 1901:421.
- pictipes** de Meijere 1911:411. To *Styloptera* (Duda 1924a:192); to *Dettopsomyia* (Duda 1926a:61).
- pictiventris** Duda 1925:211.
- pictula** de Meijere 1911:412. To *Paramyco-drosophila*.
- pilicrus** Duda 1926a:71, 74.
- pilifacies** Malloch 1926:29.
- pilimana** Grimshaw 1901:61.
- pilosula** Becker 1908:156. Duda (1935a) consid. syn. of *fasciata* Meigen.
- pinguis** Walker 1865:128. To Lauxaniidae.
- pinicola** Sturtevant 1942:40.
- plagiata** Bezzi 1908a:197.
- platitarsus** Frota-Pessoa 1954:276.
- pleuralis** Williston 1896:411. Has been consid. *Mycodrosophila*, but is prob. *Drosophila*, subgen. *Hirtodrosophila*.
- pleurofasciata** Duda 1924a:213. Syn. of *picta* Zetterstedt.

- pleurovittata** Harrison 1954:108.
plumosa Grimshaw 1901:72.
plurilineata Villeneuve 1911:83. Syn. of *busckii* Coquillett.
poecila Burla & Pavan 1953:311. N. n. for, and syn. of, *Paramycodrosophila poeciloptera* Duda 1925; see remarks *antea*.
poecilithorax Malloch 1925:87.
poecilogastra Duda 1926a:65, as var. of *latifrons* Duda.
poeciloptera (Duda 1925:226, in *Paramycodrosophila*).
poeciloptera Duda 1940:26, 34. Preocc.; replacement name is *punctatonervosa* Frey.
poeyi Sturtevant 1921:76. To *Zygothrica*.
pokorny Duda 1924a:218.
polita Grimshaw 1901:71.
pollinosa Williston 1896:414. To Ephydridae.
pollinospadix Patterson & Mainland 1944:55.
polychaeta Patterson & Wheeler 1942:102.
polymorpha Dobzhansky & Pavan 1943:19.
polypori Malloch 1924b: 351.
ponderosa Patterson & Mainland 1943:198.
prashadi Brunetti 1923:303.
preciosa de Meijere 1911:410. To *Pictostyl-optera* (Duda 1924a:192); to *Dettopsomyia* (Duda 1926a:61).
procardinoides Frydenberg 1956:60.
procnemis Williston 1896:412. To *Chymomyza*.
prognatha Sturtevant 1916: 340.
projectans Sturtevant 1916:342. To *Mycodrosophila*.
prorepleta Duda 1925:210, as var. of *repleta* Wollaston.
prosaltans Duda 1927:164.
prosimilis Duda 1927:194, as a form, not variety, of *similis* Williston ("Ich bezeichne eine Form vorläufig als **prosimilis**"); later treated as sp. by Dobzhansky & Pavan 1943: 23.
proxima Adams 1905:186. To *Leucophenga*.
pruinifacies Frota-Pessoa 1954:292.
pruinosa Duda 1940:27, 41
pseudomelanica Sturtevant 1916:333. Is prob. *putrida* Sturtevant, melanistic form.
pseudoobscura Frolova 1929:212.
pseudosaltans Magalhães 1956:273.
pseudotakahashii Mather 1957:222.
pugionata de Meijere 1915a:56.
pulchella Sturtevant 1916:327. N. n. for *bel-lula* Williston, not Bergroth.
pulchra Schiner 1868:239. To *Leucophenga* (Basden, pers. comm.).
pulchrella Tan, Hsu, & Sheng 1949:198.
pulla Pavan & da Cunha 1947:10 Syn. of *guarajá* King.
pullata Tan, Hsu, & Sheng 1949:200.
pulvera Duda 1927:181. (*pulverea* is an invalid orig. spelling).
pumilio de Meijere 1908:153.
punalua Bryan 1934:438.
punctatonervosa Frey 1954:32. Prob. syn. of *poeciloptera* Duda 1940; the latter being preocc., this is an available replacement name.
puncticeps Okada 1956:94.
punctipennis (van der Wulp 1886:56, in *Discomyza*). Syn. of *lurida* Walker.
punctipennis Duda 1940:24, 29, as var. of *bicolor*; syn. of *Lissocephala unipuncta* Malloch (Burla 1954a).
punctiscutata Lamb 1914:333. Poss. to *Neotanygastrella* (Burla 1954a).
punctulata Loew 1862:232. Syn. of *repleta* Wollaston.
pusilla Grimshaw 1901:70.
pusio Duda 1923:50.
putrida Sturtevant 1916:339.
pygmaea Duda 1926a:94, 102.
pygmaea Duda 1927:125, as var. of *repleta* Wollaston.
quadrata Sturtevant 1916:341. To *Microdrosophila*.
quadrilineata de Meijere 1911:396. To *Chaetodrosophilella*.
quadrimalculata Walker 1852:410. To *Leucophenga*.
quadrimalculata Adams 1905:182. Preocc.; n. n. is *adamsi* Wheeler.
quadripunctata de Meijere 1908:154. To *Leucophenga*.
quadriradiata Duda 1923:46.
quadriseriata Duda 1924a:211, & 1924b:255.
quadrivittata Okada 1956:83.
quadrum (Wiedemann 1830:507, in *Trypeta*).
quinaria Loew 1865:182.
quinqueannulata Frey 1917:31.
racemova Patterson & Mainland 1944:44.
ramsdeni Sturtevant 1916:328.
ramulosa Burla 1956:266.
reamurii Dufour 1845:201. Sp. inc.
rectangularis Sturtevant 1942:38.
remota Walker 1849:1111. Prob. not *Drosophila*.
repleta Wollaston 1858:117.
reticulata Wheeler 1957:101.
rioensis Patterson 1943:152, as subsp. of *meridiana*.
ritae Patterson & Wheeler 1942:87.
robusta Sturtevant 1916:331.

- rostrata** Duda 1925:219.
ruberrima de Meijere 1911:403.
rubidifrons Patterson & Mainland 1944:48.
rubra Sturtevant 1927:369.
rubrifrons Patterson & Wheeler 1942:107.
rubrostriata Becker 1908:155. Syn. of *busckii* Coquillett.
rudis Walker 1860:168. To *Leucophenga*.
rufa Kikkawa & Peng 1938:529.
ruficeps von Roser 1840:62. Sp. inc., poss. to *Scaptomyza*.
rufifrons Loew 1873:50.
rufipes Meigen 1830:87. Sp. inc., prob. to *Scaptomyza*.
rufuloventer Lamb 1914:344.
saba Burla 1954a:141.
sadleria Bryan 1938:41.
salatigae de Meijere 1914:260. To *Leucophenga*.
saltans Sturtevant 1916:328.
samoensis Harrison 1954:103.
sanyi Burla 1954a:113.
scaptomyzoptera Duda 1935a:95.
schildi Malloch 1924a:10.
schmidtii Duda 1924a:213.
scioptera Duda 1927:179.
scutellaris Duda 1929:420.
scutellata Duda 1926a:65, 70, as var. of *dentata* Duda 1924.
scutellimargo Duda 1924a:206, & 1924b:243. Nom. nud. in Duda 1923:43; consid. by Duda 1926a as var. of *brunnea* de Meijere.
segúyi Smart 1945:56. N. n. for *subobscura* Segúy, not Collin.
sellata Sturtevant 1942:39. Consid. syn. of *prosaltans* Duda but consid. prob. valid sp. by Magalhães & Bjornberg 1957:448; prob. syn. of *saltans* St.
semialba Duda 1925:208.
semiatra de Meijere 1914:265.
semiatricornis Duda 1934:63, and 1935a:69, as var. of *graminum* in *Scaptomyza* which was consid. subg. of *Drosophila*. To *Scaptomyza*.
seminigra Duda 1926a:65, 68, as var. of *latifrons* Duda; consid. sp. by Malloch 1934a.
seminole Sturtevant & Dobzhansky 1936:577.
senilis Duda 1926a:91. Type of subg. *Macropalpus*.
senufo Burla 1954a:137.
separata de Meijere 1911:406.
serenensis Brncic 1957a:78.
sericea Lamb 1914:326. To *Leucophenga*.
serrata Malloch 1927:6.
setapex Patterson & Mainland 1944:55.
setifemur Malloch 1924b:351.
setiger Grimshaw 1901:64.
setosa Villeneuve 1921:158. Syn. of *testacea* von Roser.
setosa Dobzhansky & Pavan 1943:46. Preocc.; syn. of *hydei* Sturtevant.
setula Heed & Wheeler 1957:18.
sexlineata Duda 1940:26, 36, as var. of *quadrimaculata* Adams.
sexpunctata Segúy 1938:352.
sexvittata Okada 1956:78.
sharp Grimshaw 1901:65.
sigmoides Loew 1872:103. Type of subg. *Siphlodora*.
signata Duda 1923:48.
silvata de Meijere 1916:206.
silvestris Basden 1954:618.
similis Williston 1896:415.
similis Lamb 1914:347. Preocc.; n. n. is *errans* Malloch; syn. of *ananassae* Doleschall.
simplex de Meijere 1914:266.
simulans Sturtevant 1919:153.
singularis Duda 1924a:220, & 1924b:249. Nom. nud. in Duda 1923:56.
sogo Burla 1954a:198.
solemnis Walker 1860:168.
sordida Zetterstedt 1838:777. To *Scaptomyza*.
sordidapex Grimshaw 1901:63.
sordidula Kikkawa & Peng 1938:539.
soror Schiner 1868:240. Not *Drosophilidae* (Basden, pers. comm.).
sororia Williston 1896:408.
spadicifrons Patterson & Mainland 1944:49.
spenceri Patterson 1943:160.
sphaerocera Thomson 1869:596. To *Heleomyzidae* (Malloch 1934b:437); to *Drosophilidae*, genus uncertain (Sabrosky, pers. comm.).
spinatermina Heed & Wheeler 1957:30.
spinicauda Malloch 1926:30.
spinipes Lamb 1914:336.
spinofemora Patterson & Wheeler 1942:104. Poss. syn. of *nasuta* Lamb.
splendida Williston 1896:412. To *Clastoptero-rivia*.
spurca Zetterstedt 1847:2550. Syn. of *tristis* Fallén (Frydenberg 1955:110).
stackelbergi Duda 1935a:96.
stalker Wheeler 1954:52.
sternopleuralis Okada & Kurokawa 1957:6.
sticta Wheeler 1957:96.
stonei Pipkin 1956:254.
striaticeps Duda 1923:58.
strigifrons de Meijere 1914:264.
strigiventris Duda 1927:184.
sturtevanti Duda 1927:167.
subacuticornis Duda 1924a:207, & 1924b:244.
subbadia Patterson & Mainland 1943:198.

- subfasciata** de Meijere 1914:257.
subflavohalterata Burla 1956:264.
subfunebri Stalker & Spencer 1939:108.
subgilva Burla 1956:263.
subinfumata Duda 1925:221.
sublineata Duda 1926a:65, 69, as var. of *latifrons* Duda.
submacroptera Patterson & Mainland 1943:186.
submelanica Patterson 1942a:10. Manuscript name for *melanica* Sturtevant.
subnitida Malloch 1927:5.
subobscura Collin 1936:25.
subobscura Segúy 1938:352. Preocc.; n. n. is *segúyi* Smart.
suboccidentalis Spencer 1942:61.
subpalustris Spencer 1942:64.
subpollinosa de Meijere 1914:263. To *Leucophenga*.
subquinaria Spencer 1942:59.
subsaltans Magalhães 1956:277.
subsigmoides Patterson & Mainland 1944:26. Syn. of *flexa* Loew.
subsplendens Duda 1934:70. Descr. in *Scaptomyza* which was consid. subg. of *Drosophila*. To *Scaptomyza*.
subtilis Kikkawa & Peng 1938:541.
subviridis Patterson & Mainland 1943:140.
sucinea Patterson & Mainland 1944:31.
suffusca Spencer 1943:92.
sulcata Sturtevant 1916:330. Syn. of *colorata* Walker.
sulfurigeraster Duda 1923:48. Prob. syn. of *nasuta* Lamb.
suma Burla 1954a:200.
sumatrensis Duda 1926a:73, 79.
superba Sturtevant 1916:342. To *Clastoptomyia*.
suruku Burla 1954a:107.
suturalis Wheeler 1957:100.
suzukii (Matsumura 1931:366. in *Leucophenga*).
sydneyensis Malloch 1927:5.
takahashii Sturtevant 1927:371.
tarsalis Walker 1852:412.
tarsata Schiner 1868:240.
tectifrons de Meijere 1914:263. To *Oxyptera*.
tendata Wheeler 1959, *antea*. N. n. for *dentata* Duda 1927, not 1924.
tenebrosa Spencer 1943:93.
tenuicauda Okada 1956:141.
tenuipes (Walker 1849:1112, in *Diastata*).
terminalis Loew 1863:32. To *Scaptomyza*.
testacea von Roser 1840:62.
texana Patterson 1940:219, as subsp. of *virilis*; now consid. subsp. of *americana* Spencer.
thienemanni Duda 1931:196.
thoracis Williston 1896:411.
tibialis Wheeler 1957:100.
tibudu Burla 1954a:194.
tigrina Buzzati-Traverso 1943:8. Syn. of *buzzatii* Patterson & Wheeler.
tjibodas de Meijere 1916:205.
tolteca Patterson & Mainland 1944:32.
torrei Sturtevant 1921:86.
tranquilla Spencer 1943:200.
transversa Fallén 1823:6.
trapeza Heed & Wheeler 1957:25.
trapezina Duda 1923:41.
triangula Wheeler 1949b:192.
triangulifer Lamb 1914:343.
triangulina Duda 1927:186.
trichiaspis Duda 1940:23, 31.
trifasciata de Meijere 1916:206.
trifiloides Wheeler 1957:81.
trifilum Frota-Pessoa 1954:292.
trilimbata Bezzi 1928:158.
tripunctata Loew 1862:231.
triseta de Meijere 1911:402.
trispina Wheeler 1949b:180.
tristani Sturtevant 1921:75.
tristipennis Duda 1924a:215, & 1924b:247. Nom. nud. in Duda 1923:53.
tristipes Duda 1924a:220, & 1924b:257.
tristis Fallén 1823:7.
tristriata Heed & Wheeler 1957:31.
trivittata Strobl 1893a:282.
tropicalis Burla & da Cunha 1949:302.
tsigana Burla & Gloor 1952:164.
tuchaua Pavan 1950:26.
tumiditarsus Tan, Hsu, & Sheng 1949:205.
uebe Burla 1954a:148.
umbripennis Hendel 1936:99.
undulata Grimshaw 1901:58.
ungarensis de Meijere 1911:407.
unicolor de Meijere 1914:266.
unicolor (Malloch 1934a:293, in *Hirtodrosophila*). Preocc.; n. n. is *unicolorata* Wheeler.
unicolorata Wheeler 1959, *antea*. N. n. for *unicolor* (Malloch), not de Meijere.
unimaculata Strobl 1893a:281.
uninubes Patterson & Mainland 1943:201.
unipectinata Duda 1924a:215, & 1924b:246.
unipunctata Patterson & Mainland 1943:182.
unisipina Okada 1956:129.
unistriata Strobl 1898a:580 (usual citation is 1900:636; see references).
upoluae Malloch 1934a:305.

- ussurica* Duda 1935a:74, 98, as var. of *trivittata* Strobl.
ustulata de Meijere 1908:157.
uvarum Rondani 1875:86. Consid. syn. of *melanogaster* Meigen.
valida Walker 1858:232. To *Lauxaniidae*.
varia Walker 1849:1109. To *Leucophenga*.
variegata Fallén 1823:5. To *Amiota*, subg. *Phortica*.
variegata Grimshaw 1901:57. Preocc.; n. n. is *grimshawi* Oldenberg.
varifrons Grimshaw 1901:71.
variopicta Becker 1908:156. Consid. syn. of *fenestrarum* Fallén, but Basden (pers. comm.) consid. it valid sp.
varipes Macquart 1835:550. Sp. inc., prob. to *Camillidae* (Duda 1935a).
versicolor Mather 1955:573. Syn. of *buzzatii* Patterson & Wheeler.
verticis Williston 1896:413. Sp. inc.
vibrissina Duda 1924a:219. Syn. of *confusa* Staeger.
victoria Sturtevant 1942:33. Type of subg. *Pholadoris*.
vina Burla 1954a:112.
viracochi Brncic & Santibañez 1957:70.
virgata Tan, Hsu, & Sheng 1949:203. Poss. syn. of *annulipes* Duda (Okada).
virginea Meigen 1830:84. Syn. of *fenestrarum* Fallén.
virilis Sturtevant 1916:330.
vittata Coquillett 1895:318. To *Parascapto-myza*.
vittatifrons Williston 1896:408. To *Zygothrica*.
wheeleri Patterson & Alexander 1952:129.
willistoni Sturtevant 1916:327. N. n. for *pal-lida* Williston, not Zetterstedt.
willowsi Curran 1936:42.
xanthogaster Duda 1924a:217, & 1924b:248.
xanthopyga Duda 1924a:215, as var. of *montium* de Meijere.
xanthosoma Grimshaw 1901:68.
yakuba Burla 1954a:161.
yucatanensis Spencer 1940a:160, as subsp. of *hydei* Sturtevant.
zebrina Bezzi 1928:157.
z-notata Bryan 1934:437.

SUMMARY

Eight new names are proposed to replace rejected junior homonyms in the genus *Drosophila*, and an annotated list of all known species, subspecies and varieties is presented. There are 1,024 names in the list, credited to 119 authors. At the time of writing, 920 species have been described in the genus, not including the 16 subspecies and 27 varieties. Of the 920 proposed species, 111 have been transferred to other genera, about 15 more should probably be transferred, about 70 are names of synonyms (including those listed as possible or probable synonyms), and 25 are new names proposed for rejected homonyms. Of the 23 species listed as transfers from other genera, only 13 remain as valid *Drosophila* species. There have been some changes from subspecific to specific status, and *vice versa*, and it is evident that several of the proposed varieties should probably be considered as valid species. Only a single fossil species has been described.

The list contains, therefore, the names of approximately 750 presumably biologically valid species. Only two women have been honored by having species named for them, while 87 men have been so honored. The single largest contributor to the list is Dr. O. Duda who was responsible for 16% of the proposed names, while 14.5% are due to the efforts of Prof. Patterson, his students and colleagues at the University of Texas.

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Genetic Studies of Irradiated Natural Populations of *Drosophila*.

III. Experimental Populations of *Drosophila ananassae* Derived from Irradiated Natural Populations

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INTRODUCTION

With the advent of large scale nuclear weapons tests in the Marshall Islands it became feasible to study the effects of radiation on natural populations of *Drosophila*. The first studies of this type were reported by Stone *et al.* (1957), and by Stone and Wilson (1958). In these studies, populations of *Drosophila ananassae* from some of the Marshall Islands and Eastern Carolines, which had been subjected to varying amounts of direct and fallout irradiation, were analyzed with respect to radiation effects.

The present study is an outgrowth of these earlier studies, and is designed to determine the changes in the evolutionary fitness of certain of the Marshall Island populations of *Drosophila ananassae* as measured by their response to different test conditions in the laboratory. Changes in viability, fertility, and fecundity of the populations were used as measures of changes in evolutionary fitness. Viability is defined as the percentage of eggs that develop into adults, fertility as the percentage of matings yielding offspring, and fecundity as the average number of eggs laid per day per female.

Offspring from flies that were collected in the summers of 1956 and 1957 were subjected to two different types of experimental conditions subsequent to each summer's collection. One type of condition involved maintaining the offspring of the collected flies in population cages containing from 2,000 to 10,000 adults and in which severe competition prevailed. The second type of condition involved maintaining the offspring of the collected flies in groups of pair matings in such a way that brother-sister inbreeding did not occur. Thus the pair-mated populations resembled the breeding structure of an "isolate" of a human population. The viability, fertility, and fecundity of both the population cages and pair-mated populations were checked periodically. Changes in these factors were observed and comparisons were made with populations which had received different amounts of radiation. Rates of change in the cage populations were compared to rates of change in the pair-mated populations and changes in both types of experimental populations were compared to changes in the original populations remaining in the islands.

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PROCEDURE

In the population cage tests flies from the Bikini and Majuro Atolls were used. The Bikini flies were used for the experimental population since the Bikini Atoll received more radiation than any of the other Atolls and the Majuro flies were used for the control population since the Majuro Atoll received very little radiation. Population cages were started in the following way. After each year's collection, females that had been fertilized in nature were raised in individual culture vials. F_1 from these females were transferred to half pint bottles, taking care to prevent the more fertile females from contributing disproportionate numbers of offspring. Several thousand of the F_2 were then transferred to the population cages. The population cages used in this experiment were essentially boxes screened on four sides with glass tops and wooden bottoms containing 15 evenly spaced holes. Each hole was fitted with a large cork which had a food cup fastened on top. (For a detailed description of the population cage, see Wright and Dobzhansky, 1946.) The 15 food cups were changed at the rate of seven per week so that no cup remained in a cage more than 15 days. Duplicate cages were set up both years so that two cages of Majuro flies and two of Bikini were tested each year. With duplicate cages each cage could serve as a control for the other and also as insurance against failure of the experiment through the loss or contamination of one cage.

In the tests involving groups of pair matings, flies from the Bikini and Rongerik Atolls were used in 1956 and flies from Bikini and Rongelap in 1957. The substitution of Rongelap for Rongerik in 1957 was the result of an inadequate collection at Rongerik in that year. The Bikini Atoll, as previously mentioned, received a large amount of radiation and Rongerik and Rongelap, by comparison, received intermediate amounts. Each pair-mated population was started by making 500 F_1 pair matings using the progeny from the flies collected on Bikini, Rongelap, or Rongerik. Usually between one-half and one-fourth of the 500 matings were fertile. A few progeny were taken from each fertile mating and used to make 500 matings for the next generation. Matings were made in such a way that brother-sister inbreeding did not occur. This procedure was followed in establishing each succeeding generation. Strictly speaking, there was no control for these particular tests but that did not prevent comparisons from being made between the populations in this type test and also between the populations in these tests and populations in other tests.

Both the cage populations and the pair-mated populations were maintained at 77 degrees F., which approximates the mean daily temperature in the Marshall Islands. Each experimental population was sampled at the end of three months and again at the end of six months.

To sample the population cages two food cups were introduced into each cage and allowed to remain 48 hours. They were then removed and the contents were distributed among several half pint bottles containing supplementary food. Larval competition was reduced in this manner in order to retain as many genotypes as possible. A number of offspring, selected at random from the half pint bottles were then put up in pair matings. About 150 such matings were made from which 60 were selected at random for further use. From the offspring

of each of the 60 pair matings four brother-sister pair matings were made. A similar number of offspring from each of the pair matings were crossbred to offspring of the other 59 pair matings. These crossbred matings were also put up in pairs and were made in such a way that no two groups of siblings were mated to each other. In practice only about 40 of the original 60 pair matings produced enough offspring to make both kinds of matings. Consequently the offspring from half of the unprolific parents were inbred in brother-sister matings while offspring from the other half were crossbred in order to have the unprolific parents contribute equally to the inbred and crossbred matings. The inbred and crossbred matings, when analyzed, showed the reproductive capacities of the population at their worst and best respectively.

From the above procedure 120-160 inbred matings and a like number of crossbred matings were obtained. All inbred and crossbred matings were made with flies that had been aged for six to seven days. After the matings had been made the flies were given 24 hours in which to mate and then were transferred to fresh food every 24 hours for four days. The eggs laid each day were counted and the number recorded so that when the adults emerged the percentage of eggs that developed into viable adults could be determined. First day egg counts are often omitted from the data so as to be sure that egg counts were made on inseminated females only. The matings were not discarded after the four day egg counting period but were kept for an additional ten days without being transferred. This additional ten day period allowed many matings to produce offspring when they had produced none during the four day egg laying period. Thus a more accurate estimate of the fertility of the population could be made.

To sample the pair-mated populations 60 fertile vials were selected at random from the population. There 60 were inbred and crossbred in the same way as the 60 fertile vials derived from the population cages and analyzed in the same way. The remaining fertile vials were used to continue the population.

RESULTS

The data from the crossbred and inbred test matings from the various populations are shown in Table 1 through 4 and in Figures 1 through 12. In Tables 1 and 2 the first column shows the total number of matings on which egg counts were made, and the second column shows the percentages of the matings which produced no offspring. The third column gives the number of matings that produced offspring. Columns four and five deal with the number of lethals in the population.

The fourth column gives the percentage of matings in each test in which less than 80 per cent of the eggs developed. Theoretically, in an inbred mating, if both members were heterozygous for a recessive lethal which they had received from their parents, 25 per cent of the eggs produced by this mating, neglecting other factors, would be homozygous lethal and would not develop. Thus such a mating would fall into the 75 per cent egg development class. However, if this mating falls in the 75 per cent egg development class it cannot be distinguished, in the present experiment, whether a single recessive lethal has become homozygous or whether several genes have become homozygous and

KEY TO TABLES 1 AND 2

Column 1 gives the total number of matings made in each test for the various populations.

Column 2 gives the percentage of the matings in column 1 which were sterile.

Column 3 gives the number of matings in column 1 which were fertile.

Column 4 gives the percentage of the matings shown in column 3 that carry at least one lethal or lethal equivalent—that is, fall in an egg development class below 80 per cent.

Column 5 gives the percentage of parent matings that carry a lethal as determined by the fact that at least one of the four brother-sister matings made from the parent mating carries a lethal.

Column 6 gives the total number of days on which eggs were counted in each test.

Column 7 gives the total number of eggs laid in each test.

Column 8 gives the average number of eggs laid per day per female and is obtained by dividing column 7 by column 6.

Column 9 gives the actual per cent of the eggs given in column 7 that developed into adults.

Column 10 gives the mean per cent of egg development per female and is based on angular transformations.

TABLE 1. 1956 Tests.

Type of population and type of test	1	2	3	4	5	6	7	8	9	10
Bikini pair-mated										
Sample 1 inbred	157	12.7	136	77.5		464	13243	28.5	60.1	63
Sample 2 inbred	101	31.7	69	74.0	92.1	226	4735	21.0	64.2	66
Sample 1 crossbred	168	14.7	141	44.6		389	11148	28.7	77.3	79
Sample 2 crossbred	121	22.3	86	46.5		239	4830	20.2	75.0	79
Rongerik pair-mated										
Sample 1 inbred	205	30.7	132	75.0	92.0	342	9107	26.6	64.0	66
Sample 2 inbred	99	32.3	58	76.0	90.9	135	4501	33.3	60.5	60
Sample 1 crossbred	188	25.0	112	39.3		274	7645	27.9	79.5	84
Sample 2 crossbred	136	44.9	61	47.5		161	3505	21.8	75.3	80
Bikini cage 1										
Sample 1 inbred	156	7.0	139	85.6	100	362	7567	20.9	57.4	57
Sample 2 inbred	119	16.7	95	82.0	100	216	7585	35.1	59.7	57
Sample 1 crossbred	156	8.3	99	54.4		376	4617	12.3	72.0	77
Sample 2 crossbred	121	7.4	104	36.5		274	10243	37.4	81.7	83
Bikini cage 2										
Sample 1 inbred	191	13.6	159	73.0	97.9	365	10957	30.0	64.2	66
Sample 2 inbred	164	22.0	102	71.6	90.2	270	6600	24.4	60.9	64
Sample 1 crossbred	169	23.7	124	37.8		309	7834	25.4	82.8	80
Sample 2 crossbred	130	13.1	79	53.2		205	3665	17.9	70.3	75
Majuro cage 1										
Sample 1 inbred	160	27.5	116	67.2	95.0	316	8166	25.8	64.9	69
Sample 2 inbred	126	20.6	88	76.1	97.1	219	7929	36.2	61.0	64
Sample 1 crossbred	182	27.5	99	35.4		291	4849	16.7	78.0	84
Sample 2 crossbred	118	14.4	94	24.5		245	8134	33.2	87.8	91
Majuro cage 2										
Sample 1 inbred	161	15.0	131	84.8	93.4	340	9476	27.9	64.8	65
Sample 1 crossbred	120	15.0	102	18.6		272	7245	26.6	89.1	91

that the cumulative effect of these several genes, when all are present and homozygous, is lethal. The latter case is called a lethal equivalent. It sometimes happens however that a recessive lethal, and especially a lethal equivalent, may be "leaky" or not completely lethal even when homozygous. In that case a mating in which each fly was heterozygous for a "leaky" lethal would fall in an egg development class somewhat higher than 75 per cent, perhaps as high as 78 or even 80 per cent. Further, it was shown by Stone *et al.* (1957) that even with

extensive crossbreeding of different populations the viability of many matings fell as low as 80 per cent in egg development. Thus it can be assumed that there is a general level of subvital factors in all of these populations that normally reduces viability as much as 20 per cent. Such subvital factors are often recessive lethals that are not completely recessive and, as the name implies, are not fully viable when present as a heterozygote. Each subvital may then reduce the viability by a few per cent. If there are normally enough subvitals present to reduce the viability by 20 per cent, then the reduction in viability of any mating by more than 20 per cent could easily enough be due to the presence of a recessive lethal acting as a subvital that is in excess of the normal number of sub-

KEY TO FIGURES 1 THROUGH 12

The abscissa in the following figures is divided into five egg development classes. The classes between 1 per cent and 19 per cent are plotted at 10 per cent, those between 20 and 39 per cent are plotted at 30 per cent, those between 40 and 59 per cent are plotted at 50 per cent, those between 60 and 79 per cent are plotted at 70 per cent, and the classes between 80 and 100 per cent are plotted at 90 per cent.

The ordinate shows the percentage of matings in the particular tests that fell in each of the five egg development classes.

The points of these graphs would be the midpoints of the tops of a five bar histogram if the histogram were drawn in.

Matings falling in the 80-100 per cent class are considered normal, those in the 60-80 per cent class are considered to have one or two lethals or lethal equivalents, 40-60 per cent class to have two or three, the 20-40 per cent class to have four or five, and the 1-20 per cent class has six or more lethals or lethal equivalents.

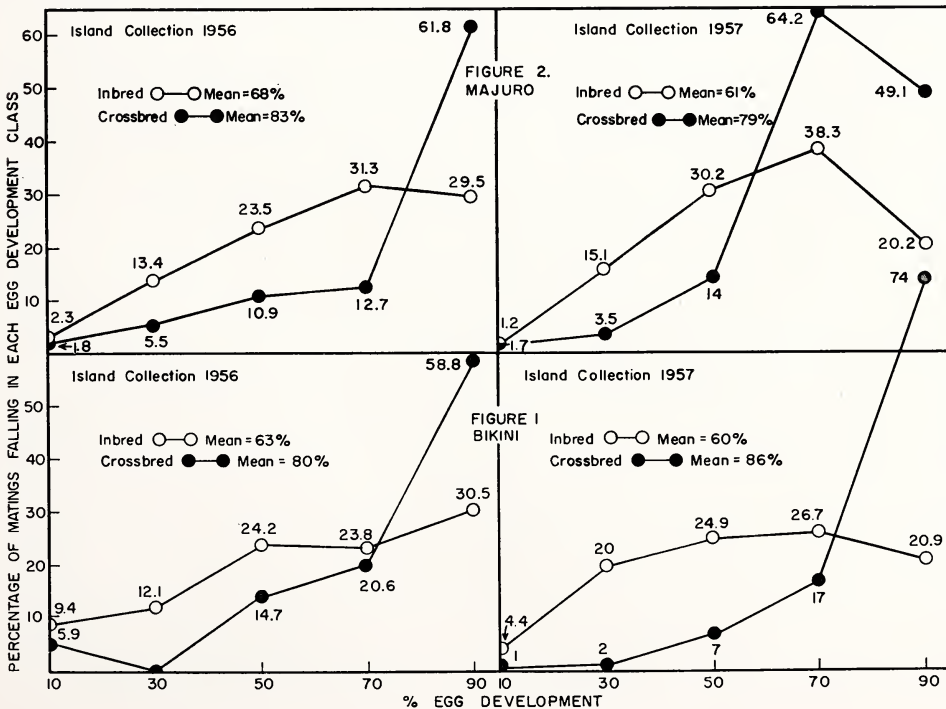


TABLE 2. 1957 Tests.

Type of population and type of test	1	2	3	4	5	6	7	8	9	10
Bikini pair-mated										
Sample 1 inbred	148	20.9	109	87.2	97.5	328	10887	33.2	48.3	48
Sample 2 inbred	150	10.7	117	81.2	97.6	239	4592	19.2	54.0	57
Sample 1 crossbred	149	20.8	108	36.1		271	6662	24.6	77.5	81
Sample 2 crossbred	138	14.5	108	38.9		279	4763	17.1	79.2	82
Rongelap pair-mated										
Sample 1 inbred	157	10.6	108	76.9	95.0	308	6303	20.5	58.6	61
Sample 2 inbred	146	25.3	87	49.4	73.5	259	2643	10.2	73.3	74
Sample 1 crossbred	142	28.9	89	56.1		251	3414	13.6	70.3	75
Sample 2 crossbred	143	21.7	94	31.9		238	3943	16.6	82.7	87
Bikini cage 1										
Sample 2 inbred	149	7.3	126	83.3	100	309	8176	26.4	59.4	63
Sample 1 crossbred	168	16.7	102	47.1		282	3605	12.8	74.5	36
Sample 2 crossbred	133	12.0	111	18.0		298	6255	20.9	86.2	89
Bikini cage 2										
Sample 1 inbred	148	17.6	101	89.1	92.3	268	3826	14.3	56.7	55
Sample 2 inbred	142	16.2	95	85.3	100	219	3026	13.8	52.2	57
Sample 1 crossbred	142	29.6	82	54.9		234	2733	11.7	73.8	77
Sample 2 crossbred	124	22.6	83	45.7		199	2777	14.0	77.2	85
Majuro cage 1										
Sample 1 inbred	110	17.3	55	70.9	77.8	176	2940	16.7	63.4	63
Sample 2 inbred	128	36.9	58	84.4	89.7	155	1543	10.0	58.5	62
Sample 1 crossbred	128	36.0	56	66.1		135	2495	18.5	62.6	67
Sample 2 crossbred	127	32.3	65	40.0		151	1446	9.6	76.8	83

vitals present. In that case the mating is said to carry a recessive lethal or lethal equivalent, even though both flies in the mating are not heterozygous for it. Lowered viability caused by a subvital lethal would be noticed more frequently in the crossbred matings than in the inbred matings because in the inbred matings such an effect would usually be masked by a more drastic reduction in viability due to the recessive lethals becoming homozygous. Therefore, the dividing line between the matings considered to be lethal free and those carrying a lethal has been placed (Stone *et al.* 1957) at 80 per cent, somewhat arbitrarily, but not without reason. The percentage of matings carrying at least one lethal is shown in column four. The analysis is further complicated by the fact that several of these various lethal factors may be present at once. The presence in a mating of the normal level of subvital factors, plus a recessive lethal, may reduce the viability to as low as 60 per cent. The presence of two lethals will not reduce the viability twice as much as one lethal because there will be some duplication of "killing" by each lethal. Thus each additional lethal has less effect. To aid in the analysis, five egg development classes were established: 80–100 per cent was considered normal, 60–80 per cent was considered to have one or two lethals or lethal equivalents, 40–60 per cent to have two or three, 20–40 per cent to have four or five, and 1–20 per cent to have six to eight or more. These egg development classes, although subject to error, are nevertheless a consistent standard whereby viability levels in different populations can be compared. The number of matings in various egg development classes for the various experimental populations for the two years are given in Tables 3 and 4. These data are plotted for ease of comparison in Figures 2 to 12. Figures 1 and 2 give data from Stone *et al.* (1957) and from Stone and Wilson (1958). They show similar plots from the

Bikini and Majuro populations on the islands in 1956 and 1957 and have been included for comparison.

Returning to the explanation of Tables 1 and 2, the fifth column shows the percentage of parent matings carrying at least one lethal. The frequency of these lethals in the P_1 generation can be determined by making brother-sister inbred matings among the F_1 . If one of the four brother-sister matings falls in an egg development class lower than 80 per cent, at least one of the parents is heterozygous for a recessive lethal. It should be noted that even if all four of the F_1 brother-sister matings fall in egg development classes above 80 per cent, there is still a 32 per cent chance that the parent mating carried a lethal which was not present in both flies in any of the brother-sister matings and consequently was not detected. Also significant is the fact that when fewer than four brother-sister matings are fertile, there is a greater chance of "missing" lethals which were present in the parent mating. Consequently the figures in column five underestimate the actual number of lethals present. Columns six, seven and eight show the total number of days on which eggs were counted, the total number of eggs laid, and the average number of eggs laid per day per female, respectively. The latter column shows the fecundity of the various populations and is obtained by dividing column seven by column six. Column nine gives the actual percentage of eggs that developed into adults and column ten gives the mean percentage of egg development per female. The latter column was calculated by converting the percentage development of each mating to the corresponding angular transformation, finding the angular transformation mean, and re-converting this to per cent. The use of the angular transformations placed more

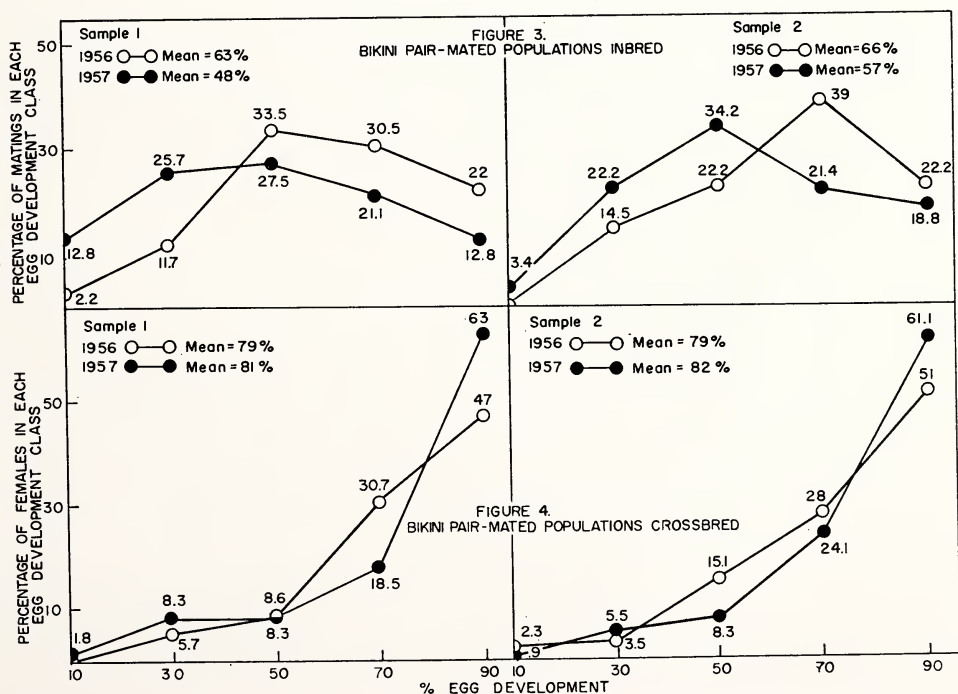


TABLE 3. 1956 Tests.

Numbers of Matings in the Various Egg Development Classes for the Various Tests

	1-9%	10-19%	20-29%	30-39%	40-49%	50-59%	60-69%	70-79%	80-89%	90-100%
Bikini pair-mated										
Sample 1 inbred	0	3	4	12	19	27	22	20	13	17
Sample 2 inbred	0	0	7	3	8	8	9	18	9	7
Sample 1 crossbred	0	0	2	6	2	10	17	26	40	37
Sample 2 crossbred	0	2	0	3	4	9	10	14	16	28
Rongerik pair-mated										
Sample 1 inbred	0	3	8	9	15	23	21	18	15	19
Sample 2 inbred	1	1	5	6	7	9	7	7	9	6
Sample 1 crossbred	0	0	1	1	6	3	17	16	31	37
Sample 2 crossbred	0	1	0	1	1	6	10	10	15	17
Bikini cage 1										
Sample 1 inbred	3	4	6	11	25	29	26	15	14	6
Sample 2 inbred	1	1	8	8	21	11	16	11	14	3
Sample 1 crossbred	1	1	1	6	7	7	14	19	21	26
Sample 2 crossbred	0	0	0	0	4	7	13	14	31	35
Bikini cage 2										
Sample 1 inbred	0	4	4	16	15	22	30	25	17	26
Sample 2 inbred	0	2	4	12	12	23	11	9	15	13
Sample 1 crossbred	0	0	1	2	6	8	9	24	38	43
Sample 2 crossbred	0	1	1	1	9	8	10	12	17	20
Majuro cage 1										
Sample 1 inbred	0	3	8	13	10	11	15	18	10	28
Sample 2 inbred	0	0	4	8	15	14	14	12	9	12
Sample 1 crossbred	1	2	1	2	4	8	7	10	22	42
Sample 2 crossbred	0	1	1	0	0	6	5	10	9	62
Majuro cage 2										
Sample 1 inbred	0	0	6	8	32	27	19	19	9	11
Sample 2 inbred										
Sample 1 crossbred	0	0	1	1	1	3	7	6	14	70
Sample 2 crossbred										

stress on the intermediate percentage values usually obtained from larger number of eggs and less stress on high and low percentages usually obtained from smaller numbers of eggs. Therefore the errors which are frequently accumulated when percentages based on different number of eggs are averaged were reduced.

The reason for including column ten was that in addition to the overall per cent of egg development shown in column nine, an important factor in the reproductive capacity of a population is the percentage of females in the population that fall in high egg development classes. That is, a population in which 50 per cent of the females fall into egg development classes below 40 per cent is not very efficient reproductively, while a population in which 90 per cent of the females fall into egg development classes above 80 per cent is considerably more efficient. Thus the mean percentage of egg development of the individuals in a population shows a slightly different picture of the reproductive capacities of the population than the overall actual percentage of viability. The mean percentages shown in column ten are the same means that appear in Figures 3 to 12, since the figures are based on the percentage development of the individual matings rather than on the actual percentage development.

In 1956 one Majuro population cage became contaminated with *Drosophila melanogaster*. This contamination was insignificant at the time of the first sample but became so severe that a second sample was not taken. In 1957 both Majuro cages were contaminated four weeks after they were started. In order to save

them, the flies were trapped out of the cages, etherized, and the *melanogaster* discarded. The food cups were put in half pint bottles to hatch and the emerging adults were separated and the *ananassae* returned to the cages. This procedure cleared up one cage which soon became as densely populated as the others. The second cage, apparently not entirely cleared of *melanogaster*, was recontaminated and lost. The number of flies in the first cage was reduced to two or three thousand at the time the flies were reintroduced. Thus this cage was run through a population bottleneck that the others were not subjected to. This may have made some difference and should be kept in mind when inspecting the data. Both the second sample of the 1956 Majuro cage and the 1957 Majuro cage which was lost are omitted from the tables. Also omitted from the tables is the first sample of Bikini cage 1 in 1957. This sample was spoiled by bad food.

The fertility of the matings in these experiments showed considerable variation as can be seen from Tables 1 and 2. The only consistent pattern in this variation was that, as in other tests (Stone *et al.* 1957), the Bikini flies were consistently more fertile than any of the other populations. This high fertility was maintained in both types of tests. Although there is usually a difference in fertility between crossbred and inbred matings in this case the averages of both types of matings showed about the same degree of fertility, the crossbred being more fertile sometimes and the inbred at others. This variability in fertility was shown when second samples were compared to first samples; at times the second sample was more fertile and at other times the first sample was more so. Also, there was no evidence to indicate an improved fertility from one year to the next.

Such variability among the various matings is not surprising in view of the

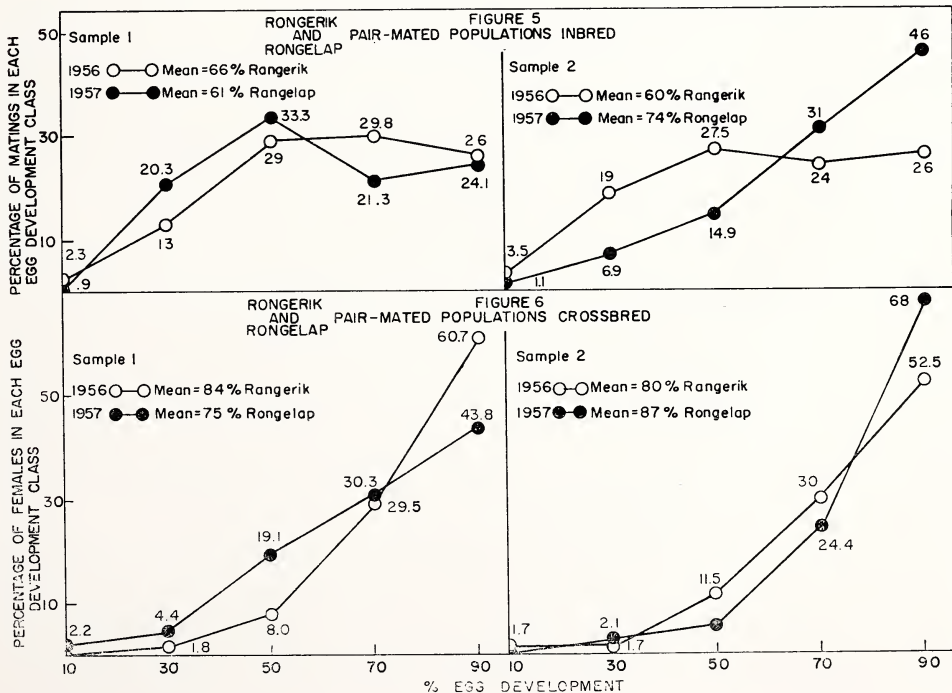


TABLE 4. 1957 Tests

Numbers of Matings in the Various Egg Development Classes for the Various Tests

	1-9%	10-19%	20-29%	30-39%	40-49%	50-59%	60-69%	70-79%	80-89%	90-100%
Bikini pair-mated										
Sample 1 inbred	3	11	12	16	15	15	15	8	11	3
Sample 2 inbred	1	3	5	21	18	22	10	15	17	5
Sample 1 crossbred	0	2	6	3	2	7	3	17	34	34
Sample 2 crossbred	0	1	1	5	5	4	8	18	32	34
Rongelap pair mated										
Sample 1 inbred	0	1	6	16	15	21	18	5	4	12
Sample 2 inbred	0	1	2	4	6	7	10	17	15	25
Sample 1 crossbred	0	2	2	2	6	11	11	16	19	20
Sample 2 crossbred	0	0	6	2	2	3	8	15	22	42
Bikini cage 1										
Sample 1 inbred										
Sample 2 inbred	0	2	4	16	14	21	25	24	12	9
Sample 1 crossbred	2	2	4	3	3	6	15	12	27	28
Sample 2 crossbred	0	0	1	0	1	2	6	11	33	57
Bikini cage 2										
Sample 1 inbred	0	3	11	12	16	19	15	14	7	4
Sample 2 inbred	0	5	13	7	5	23	9	18	10	5
Sample 1 crossbred	0	1	2	0	3	11	14	15	14	22
Sample 2 crossbred	0	1	2	4	1	5	12	14	14	30
Majuro cage 1										
Sample 1 inbred	0	1	2	10	7	5	11	3	7	9
Sample 2 inbred	0	1	1	7	6	13	10	11	5	4
Sample 1 crossbred	0	0	6	2	5	8	10	7	13	7
Sample 2 crossbred	0	2	4	2	2	3	3	11	14	24

fact that *ananassae* is a poor laboratory stock. In 1956 there was a considerable difference between the cages and the pair-mated populations in average percentage of fertility. Such a difference was not present in 1957. Also, in 1956 the pair-mated populations were themselves much more variable in fertility. This may have been due to the fact that the population size was somewhat smaller and more variable in size in 1956 than in 1957. The considerable increase in sterility between the first and second samples of the 1956 pair-mated populations probably accounts, at least in part, for the noticeable overall difference between the cages and pair-mated populations in 1956.

A few consistent differences were apparent in the percentage of lethals present in the various populations. However, none of these differences were very large. A strong case can be made for assuming that all of the F_1 matings, as shown by inbreeding tests, carried at least one lethal. As mentioned previously, even if all four bother-sister inbred matings from each of the parent matings were fertile and in egg development classes above 80 per cent, 32 per cent of the lethals in the parent matings would have been missed. Actually, enough sterility occurred so that almost always fewer than four and sometimes only one inbred mating was fertile. Consequently, considerably more than 32 per cent of the lethals carried by the parent matings were missed.

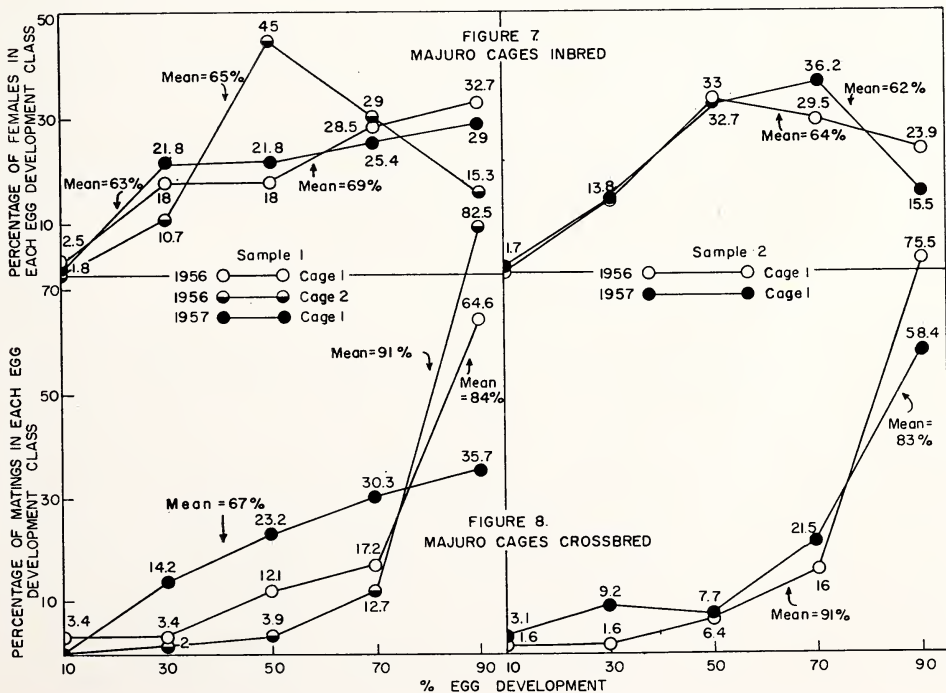
There was a consistent tendency for lethals to be eliminated between the time of the first sample and the time of the second. This indicates that there was some selection against the lethals in these tests. One should also notice, however, that the level of lethals in these tests is higher than the level of lethals found in the island populations at the time of the 1956 and 1957 collections. This suggests that

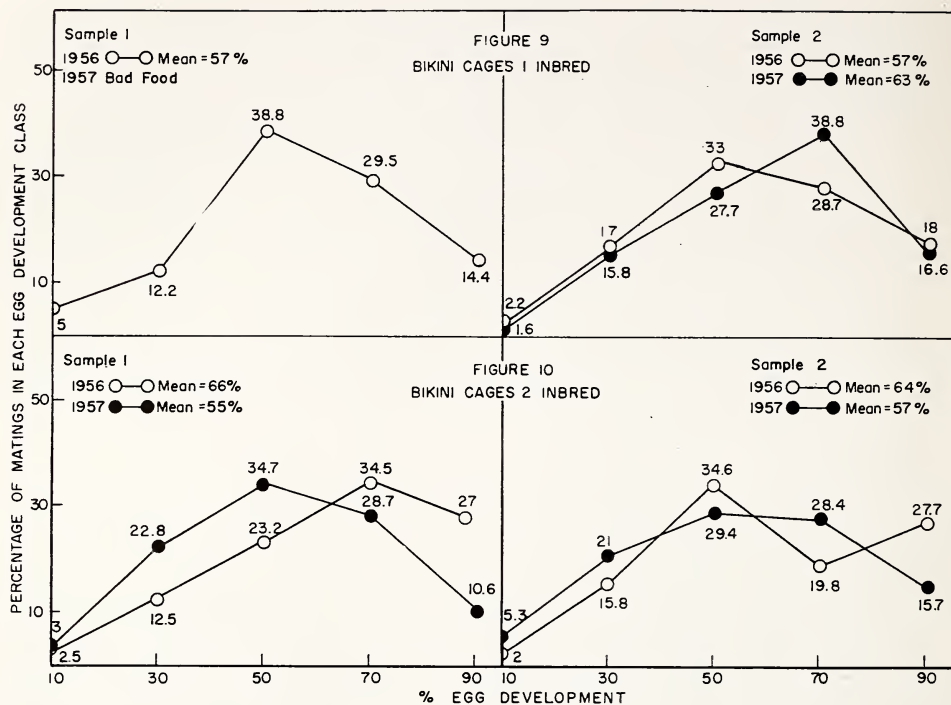
there was an initial increase in the number of lethals in the experimental populations. This was perhaps due to some inbreeding for a few generations which lasted until the population size had built up.

Viability is closely related to the number of lethals in a population although it is affected by other factors as well. In these experiments the mean viability was divided into five classes and is plotted in Figures 1 to 12. The percentage of individuals in each test whose egg development fell between 80 and 100 per cent is plotted at 90 per cent. The percentage of individuals in each test whose egg development fell between 60 and 80 per cent is plotted at 70 per cent, and so on. It can be seen that the points on the graphs would be the midpoints of the tops of five histogram bars if the histograms were drawn in. These graphs give a more accurate picture of the viability and the reproductive capacities of the various populations than one gets by merely determining the number of flies that carry at least one lethal.

The differences in viability between the various populations are also shown in Tables 1 and 2. It can be seen in each case, with the exception of Majuro cage 1 sample 1, 1957, that the inbred matings were significantly less viable than the crossbred matings. The Majuro cage had been reduced in numbers to about 2,000 from the *melanogaster* contamination only a few generations before sample 1 was taken. This probably accounts for the similarity in viability between the crossbred and inbred matings in that particular sample.

The differences between the viabilities of the crossbred matings are larger than the moderate differences between the inbred matings. The single exception to the small differences between the first and second samples in the inbred matings is





the Rongelap 1957 population. It must be noted that this population, at the time of the first sample, showed much lower viability than the corresponding parent population collected on the island three months earlier. Therefore, the considerable increase in viability between the time of the first sample and the time of the second sample merely restored the viability to a range comparable to that in the other populations.

It can also be seen from Tables 1 and 2 that fecundity, as measured by average egg production, is variable. This, as is the case with fertility, is not surprising in light of the fact that *ananassae* is a poor laboratory stock. There is often a decrease in fecundity between the first and second samples.

DISCUSSION

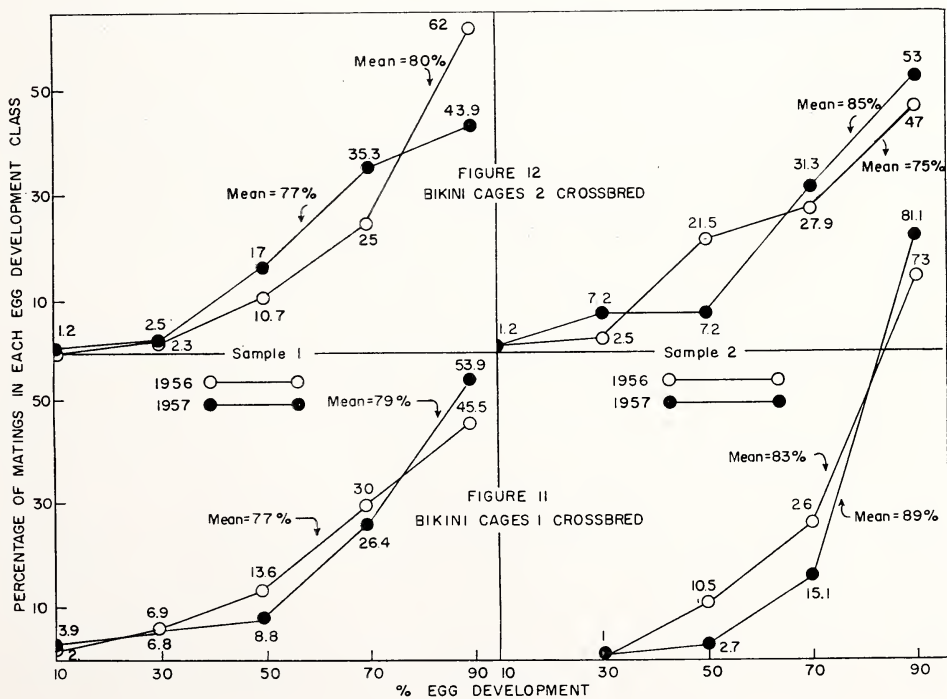
This experiment marks the first time that natural populations which received large amounts of radiation were tested under experimental conditions in the laboratory.

Wallace (1950), and Wallace and King (1951) tested in population cages various populations of *Drosophila melanogaster* which had been irradiated in the laboratory. In their tests they started with populations with lethal free second chromosomes and only studied the effects of radiation on the second chromosome of the populations. One population containing 10,000 flies was given a single large dose of radiation, one was kept as a control, and others of various sizes were subjected to chronic gamma radiation. In the heavily irradiated population there was an immediate increase in lethal frequency due to the radiation treatment.

The lethal level then fell off rapidly until the lethal level of the irradiated population was only about eight per cent higher than the level in the control population. From this point on, lethals accumulated at the same rate in the control and irradiated populations and both populations were apparently approaching equilibrium values. The population which received a single large dose of radiation apparently incorporated the radiation produced lethal mutations and other radiation produced variability, as well as spontaneous mutations, into heterotic gene combinations which increased the estimated adaptive value of the irradiated population over that of the control, for a time at least. The chronically exposed populations showed a decrease rather than an increase in adaptive value.

Such possible benefit from single large doses of radiation would not be expected to prevail in a natural population however, since the total variability of the population, including lethal mutations, is already near an equilibrium value rather than below it. Indeed, as Stone *et al.* (1957) showed, the viability of *ananassae* populations on the Bikini and Rongelap Atolls was still seriously impaired 18 months after the time of irradiation from the thermonuclear device exploded March 1, 1954, due partly at least, to an abnormally high level of lethals.

In these experiments using *Drosophila ananassae* sufficient markers and inversions were not available to enable one to make a detailed analysis of the effects of radiation on a particular chromosome of the population such as Wallace made. The complex problem of comparing the fitness of various populations was reduced to comparing the viability, fertility, and fecundity, of the various populations and, associated with viability, the lethal level. These tests made no distinction between chromosomes, but rather tested the genome as a whole. Sex linked



lethals and lethals associated with rearrangements, such as translocations and inversions, that were produced by the initial irradiation, would not be expected to be present in the populations by the time that these experiments were started. Cytological observations confirmed this expectation (Stone *et al.* 1957).

The cage populations consisted roughly of 8,000 to 10,000 flies. The size of the populations would be expected to be rather constant since the rate of food addition was constant. In contrast the pair-mated populations were quite small, consisting of at the most 1,000 flies per generation. Further, the size of the pair-mated populations was variable. Fluctuations in fertility and fecundity, especially in 1956, sometimes made it difficult to get enough flies to make 500 new matings without using large numbers of flies from the more fertile matings. From the small population size of the pair-mated populations it might be expected that some of the changes in viability, fecundity, and fertility were due in part to genetic drift. Also, the smaller populations would be expected to have been somewhat more inbred than the larger populations even though direct brother-sister inbreeding did not occur.

From the inherent differences in the two types of populations it might be expected that there would have been greater changes in one of the populations. In these experiments however, this was not usually so, although occasionally the small populations were more variable. Over a longer period of time more persistent differences might have arisen between the two populations. In this study the cages were only maintained for about 15 generations and the pair-mated populations for 12. The generation time of the pair-mated populations was extended several days by the handling time necessary for making up the matings.

The importance of the present tests lies in the fact that they serve as part of the control for the original experiments of Stone *et al.* (1957). The purpose of the original tests was to determine the effects of radiation on natural populations of *Drosophila ananassae*. The greatest problem which was confronted was that there had been no chance to study the populations before the irradiation occurred. The normal population cycles could not be measured until after the genetic damage had been done. It was necessary to study the damaged populations first and to try to establish what were normal population fluctuations later. The mean viabilities of the Majuro, Bikini, and Rongelap populations, when inbred, in 1955 were 56.6, 46.4, and 40.8 per cent respectively. By 1956 they had improved to 66.4, 60.3, and 70.9 per cent. The one small experimental pair-mated population of Rongelap flies in 1957 was the only test population that, when inbred, approached the improvement shown by the original populations on Bikini and Rongelap. The viability of this population changed from 58.6 per cent in the first sample to 73.3 per cent in the second sample. It should be noted, however, that in addition to its small size, this population was derived from the Rongelap population which recovered from a mean viability of 40.8 per cent in 1955 to 78.9 per cent in 1956. Thus it is not surprising that this pair-mated population attained a relatively high level of viability. Another point of interest is that the lowest viability shown by any population in these experiments, except for the small inbred 1957 Bikini pair-mated population, is 57.4 per cent. This lowest value is strikingly and significantly higher than the 46.6 per cent and 40.8 per cent viabilities encountered in the Bikini and Rongelap populations in 1955. The

48.3 value for the first sample, inbred, of the 1957 Bikini pair-mated population is the only value not significantly higher than the 46.6 per cent shown by the 1955 Bikini population on the atoll. This low value may be explained by supposing, as Stone and Wilson (1958) suggest, that the Bikini population has still not completely recovered and that the 1957 Bikini pair-mated population was more highly inbred than the 1956 Bikini pair-mated population.

SUMMARY

1. Samples from natural populations of *Drosophila ananassae* from the Bikini, Rongelap, and Rongerik Atolls, which had been subjected to large amounts of radiation, and a sample from the Majuro Atoll, which had received a negligible amount of radiation by comparison, were maintained in the laboratory in two types of experimental populations.

2. The various experimental populations were tested periodically for changes in viability, fertility and fecundity. These factors were used as an index of evolutionary fitness. The procedure used in testing for changes in these factors is discussed.

3. The only consistent observation as regards the fertility of the populations was that the Bikini populations were almost always more fertile than the other populations. Stone *et al.* (1957) also found the Bikini population to be more fertile than the other populations.

4. No consistent differences in fecundity were observed between the various populations.

5. Moderate decreases in the level of lethal mutations from the first sample to the second sample in most of the populations indicates that there was some selection against lethals, especially when the initial level of lethals was high.

6. That only moderate changes in viability occurred in the various experimental populations, especially in the inbred tests, is taken as strong evidence that the marked improvement of the Bikini and Rongelap populations in the Marshall Islands (Stone *et al.* 1957) between 1955 and 1956, was due to the fact that in 1955 both populations were considerably less viable than normal, due to the effects of the radiation that they had received 18 months earlier.

ACKNOWLEDGMENT

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Genetic Studies of Irradiated Natural Populations of *Drosophila*. IV. 1958 Tests¹

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INTRODUCTION

Since Muller (1927) discovered that X-rays produced mutations and chromosomal abnormalities, the problem of the effect of widespread irradiation of populations has been of great interest to scientists. The discoveries that led thereto and the development of the atomic and thermonuclear bombs have made this problem of acute interest not only to scientists but to the general human population. To a number of scientists and to the members of the Division of Biology and Medicine of the Atomic Energy Commission, it seemed to be imperative that natural populations subject to direct and fallout radiations from atomic and thermonuclear explosions should be investigated. We have been studying the genetic damage and its decay through time using natural populations of *Drosophila ananassae*. The irradiated populations live in the Pacific Proving Ground on the northern Marshall Islands, and the control populations are from Majuro in the southern Marshalls and Ponape in the eastern Caroline Islands.

In earlier publications (Stone, Wheeler, Spencer, Wilson, Neuenschwander, Gregg, Seecof and Ward, 1957; Stone and Wilson, 1958) we have reported investigations of the populations collected in the summers of 1955, 1956 and 1957. As we indicated and demonstrated in the earlier publications, *Drosophila ananassae*, a tropical species found around the world, is the characteristic species of the Pacific Islands. It is suited by its temperature tolerance and the superior competitive ability of its larvae to survive in these climates on the small islands. Ponape has several other species but on the low islands, at least, *ananassae* is the most numerous *Drosophila* species. The islands in the Pacific Proving Ground which received direct irradiation and fallout (Bikini) or fallout only (Rongelap, Rongerik, and Eniwetak in the Rongerik atoll), have populations of *ananassae* of sufficient size to be studied. The thermonuclear device of March 1, 1954, gave very heavy radiation and fallout to these areas. Since then, the fallout on Bikini island and especially on Rongelap and Rongerik has been much less intense than from the 1954 device. In fact, the Division of Biology and Medicine of the AEC informs us that probably 99% of the total fallout on Rongelap and Rongerik came from the March 1, 1954 test. The large island, Bikini, in the atoll of that name, received fallout on several other occasions. Populations of *Drosophila* have been collected from these islands in late July and August of 1955, 1956, 1957 and 1958. This paper reports the tests of the *ananassae* populations collected in 1958.

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EXPERIMENTAL

The populations collected on the several islands were shipped air mail to the University of Texas for analysis. Unfortunately, the size of the sample which reached Austin for analysis was small this year. On Ponape a typhoon had reduced the fruit to such a low level that only one very small local population was sampled. The population on Majuro was low also, as was the one on Rongelap where freshly fallen fruit was very difficult to find. The F_1 generation from the individual females or pairs sent in was not large enough and an F_2 from crosses of progeny of different females had to be raised in the laboratory to secure enough flies for the tests. These were the flies used in tests 1 through 15, Table 1. The progeny of crosses 10 through 15 were tested in the crosses 16 through 26.

In order to assay the genetic and environmental components of variation in these populations, we compared the results of crossbreeding (crossing offspring of different parents within a population or crossing individuals from different populations) and inbreeding (brother $\times$ sister matings). The variables tested are basic components in natural selection: fertility, fecundity and viability. The pairs were mated five to eight days after emergence to insure their sexual maturity. Fertile pairs are those which produced eggs that developed. Viability was measured in terms of egg development. After mature pairs were allowed to mate one day, they were changed daily for four or five days to fresh food, the number of eggs laid on each day was recorded, then the number of adults that developed was determined after a suitable interval of 12 to 15 days. After the egg counts the pairs were placed on fresh food. These vials were examined at the end of the period to determine if any pairs had proven to be fertile although no eggs from the egg count period had developed. More detailed accounts of the techniques and methods of analyses are to be found in the article by Gregg (1959, this bulletin).

RESULTS

The data from the tests of the populations collected in 1958 are given in Table 1. The first four columns give the tests for fertility and an estimate of the number of pairs in which at least one lethal was present. This could be determined for the parents of brother-sister matings but since too few such cultures were tested and fertile, the number of pairs with one or more lethals is underestimated.

The next four columns of Table 1 give the fecundity, measured as the average number of eggs laid per day, and the percentage of pairs that showed the presence of a lethal or lethal equivalent. If the egg development from a pair was less than 80%, the pair was scored as having one or more lethals or lethal equivalents. The last seven columns give the information on total egg development from a cross and the pattern of egg development for each cross. The last five columns, giving the pattern of egg development, were used to make the comparisons between the results in 1958 and in earlier years shown in Figures 1 through 4.

The data can be compared between populations, between years, and within and between populations and their heterozygotes using sib matings and crossbreeding. Environmental effects are always present and it is desirable to discriminate as much as possible between environmental effects and genetic effects on the variables measured.

TABLE 1
Results of crosses involving material collected in 1958*

Test	P ₁ Females			F ₁ Pairs			Egg Development									
	No.	Per Cent with Lethals	Pairs Tested	Per Cent Sterile	No. Fertile	Per Cent Lethals	Days Eggs Counted	Eggs/day/♀	Total Eggs	Per Cent Developed	Pattern (Per Cent)					
											1-19	20-39	40-59	67-79	80-110	
1. Ponape (P)	32	75.0	136	33.1	71	76.1	179	29.9	5,357	63.4	1.4	12.7	28.2	33.8	23.9	
2. P (random)	..	...	130	44.6	68	41.2	213	24.4	5,200	81.5	..	1.5	10.3	29.4	58.8	
3. Majuro (M)	15	93.3	76	52.6	29	86.2	94	23.7	2,224	50.3	..	34.5	38.0	14.8	14.8	
4. M (random)	..	...	118	40.7	55	49.1	162	21.2	3,439	69.8	7.3	7.3	10.9	23.6	50.9	
5. Bikini (B)	46	87.0	187	40.6	93	76.3	236	26.0	6,128	65.1	..	9.7	25.8	40.9	23.7	
6. B (random)	..	...	169	42.6	84	57.1	230	20.0	4,609	74.7	1.2	3.6	20.2	32.1	42.9	
7. Rongerik	18	100.0	85	50.6	20	75.0	83	20.0	1,660	55.2	5.0	10.0	30.0	30.0	25.0	
8. Rongelap (A)	25	80.0	110	44.5	47	74.5	125	22.4	2,801	61.2	..	6.4	34.0	34.0	25.5	
9. A (random)	..	...	137	31.4	82	52.4	210	17.2	3,602	73.5	..	8.5	8.5	35.4	47.6	
10. M × P	..	...	165	49.7	73	41.1	237	21.8	5,158	77.1	..	2.7	8.2	30.1	58.9	
11. A × M	..	...	151	25.8	86	46.5	286	17.0	4,861	71.1	1.2	4.7	15.1	25.6	53.5	
12. M × B	..	...	127	44.9	54	53.7	136	20.2	2,749	73.6	3.7	9.3	7.4	33.3	46.3	
13. P × A	..	...	181	93.4	9	33.3	22	25.9	570	63.9	..	11.1	11.1	11.1	66.6	
14. B × P	..	...	126	41.3	57	54.4	185	19.8	3,658	73.8	..	1.8	12.3	40.4	45.6	
15. B × A	..	...	105	28.3	67	83.6	165	38.5	6,359	60.0	1.5	7.5	31.3	43.3	16.4	
16. AM × BP	..	...	116	25.9	82	51.2	257	23.4	6,018	76.2	..	...	7.3	43.9	48.8	
17. BA × MP	..	...	126	65.9	40	50.5	120	22.4	2,689	75.9	2.5	2.5	5.0	40.0	50.0	
18. MB × PA	..	...	99	41.4	39	53.8	107	18.8	2,015	70.2	2.6	12.8	12.8	25.6	46.2	
19. AM × AM	44	100.0	172	34.9	108	83.3	317	26.7	8,463	57.6	1.9	16.7	33.3	31.5	16.7	
20. BA × BA	16	100.0	108	53.7	30	93.3	75	22.4	1,677	42.0	..	46.7	30.0	16.7	6.6	
21. MB × MB	30	93.3	110	26.4	70	87.1	167	23.4	3,914	57.4	4.3	22.9	28.6	31.4	12.9	
22. BP × BP	36	97.2	192	56.3	63	90.5	147	24.5	3,611	57.7	3.2	20.6	33.3	33.3	9.5	
23. MP × MP	34	91.2	156	53.8	63	87.3	167	29.0	4,841	53.9	4.8	27.0	25.4	30.2	12.7	
24. PA × PA	9	100.0	32	25.0	20	80.0	65	32.2	2,093	62.0	..	10.0	30.0	40.0	20.0	
25. MB × BA	..	...	123	17.8	93	63.4	267	23.2	6,281	64.8	8.6	8.6	17.2	29.0	36.6	
26. AM × MB	..	...	120	33.3	79	67.1	254	23.3	5,922	71.8	2.5	3.8	12.7	48.1	32.9	

* Given at the left are the strains, their letter symbols, and crosses. Column 1 gives the number of females which gave fertile progeny as demonstrated in the brother-sister matings (cols. 3-5). If one or more of these progeny pairs had a lethal or lethal equivalent genetic complex (scored lethal when less than 80 per cent of the eggs laid by a female which produced progeny developed), their parent pair was scored as having one or more lethals in column 2. "Per Cent with Lethals" (the per cent showing one or more lethals in column 2) give the number of eggs laid by females known to have been fertilized and the per cent of these eggs that developed into adults. The pattern of egg development in the several crosses is shown in columns 11-15. These columns give the per cent of females in classes with their egg development ranging from 1 to 19 per cent, etc. Only the last class (80-100 per cent) is regarded as normal development. The results are shown diagrammatically in Figs. 1-4.

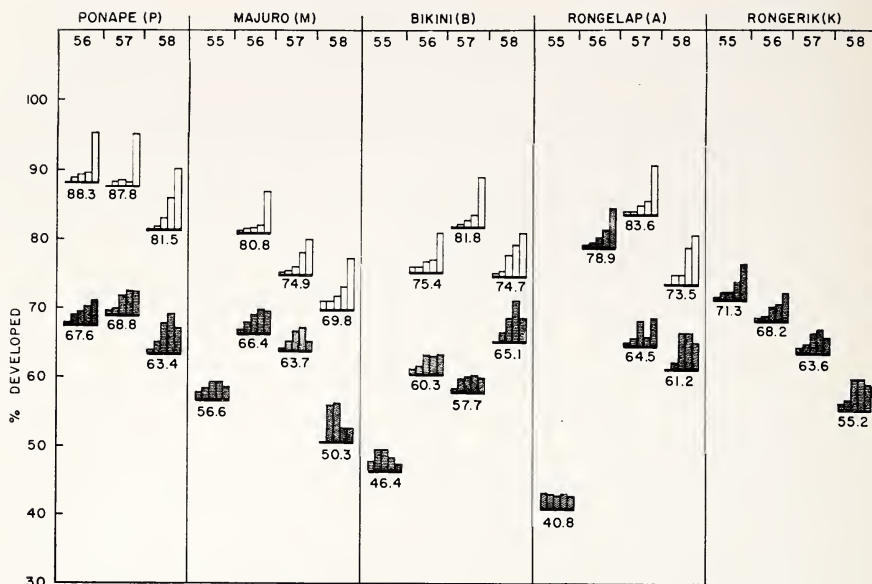


FIG. 1. Egg development within populations. The darkened figures are brother $\times$ sister matings; the plain figures are crosses between offspring of different pairs. These were run at the same time, with the progeny of pairs tested both ways when possible. The test years are indicated at the top for each population. The histogram shows the relative percentage of females with egg development of 1-19, 20-39, 40-59, 60-79, and 80-100 per cent from left to right. The frequency distribution of females which gave different effective egg developments gives a better picture of the effective productivity of the population or cross. The average per cent development of all eggs laid is given under each population histogram. The letter symbol for the particular island is indicated with the name.

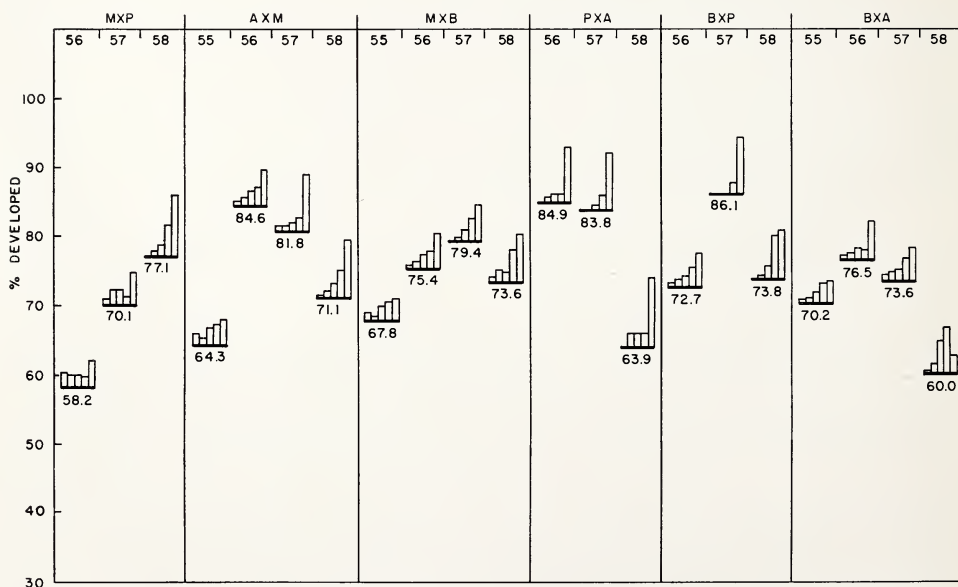


FIG. 2. Distribution of classes and average egg development for crosses between flies from different populations.

The Ponape (P) population was not sampled in 1955. Ponape is a larger high island with a number of species in competition. *Drosophila ananassae* populations from Ponape are slightly superior in egg development on inbreeding or cross-breeding to the other populations sampled, Figure 1. The crosses and heterozygotes of Ponape with other strains do not differ very much from other tests

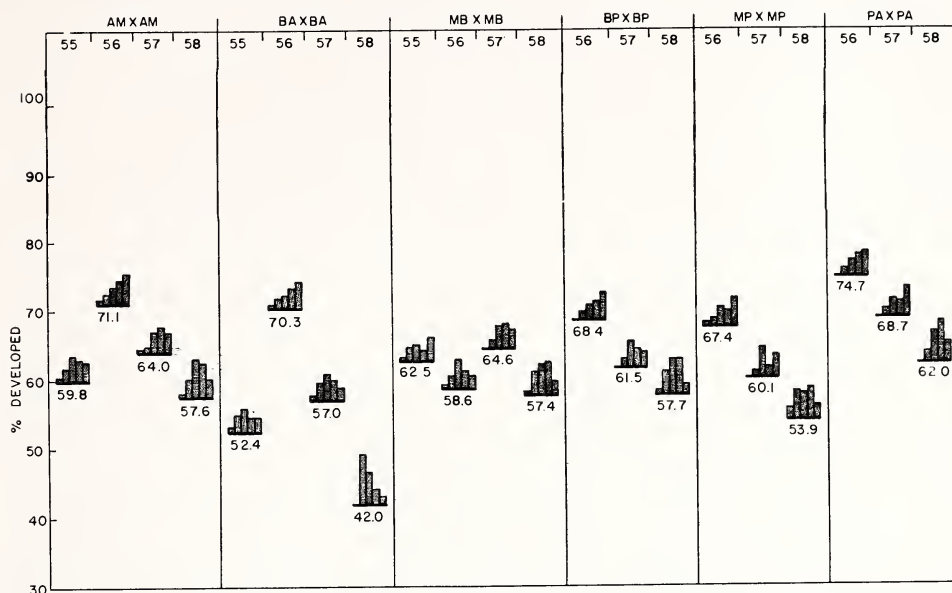


FIG. 3. Distribution of classes and average egg development for brother $\times$ sister matings of F_1 from crosses between populations.

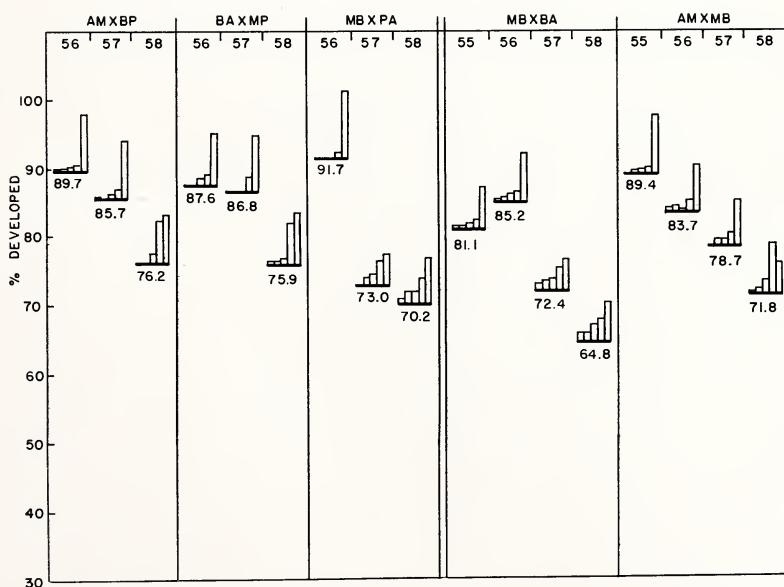


FIG. 4. Distribution of classes and average egg development for three-way and four-way crosses between different F_1 's.

although there is an indication that flies from Ponape do not cross as readily to the other strains as they do among themselves. There may be some sexual isolation factors acting to separate these populations. The strains from Majuro (M), Bikini (B), and Rongelap (A) have been tested each year. In following the crosses of these populations by numbers in Tables 1 and 2, tests 3, 5 and 8 are inbreeding tests using brother $\times$ sister matings, 11, 12 and 15 are crosses between these strains, 19, 20 and 21 are brother $\times$ sister matings of the F_1 heterozygotes between populations, while 25 and 26 are crosses between these heterozygotes, termed "three-way" crosses because they involve three strains. Before considering Table 2, it is well to compare the several variables measured in 1957 with 1958 for all tests. Differences in the unweighed averages of all tests in 1957 and in 1958 showed a decrease in average egg development of 7.3% (72.8 to 65.5%), 2.2% increase in sterility (40.1 to 42.3%) and reduction in average number of eggs laid per day of 3.6 (27.3 to 23.7). The simplest assumption is that the laboratory environment was less favorable in 1958, especially in view of the consistency of the results—for example, the per cent egg development decreased in 26 of 28 tests.

The values and their changes for egg development for all stocks and crosses tested each year from 1955 to 1958 are given in Table 2. The general trend is for

TABLE 2
Differences in egg development*

Tests	1955	Difference	1956	Difference	1957	Difference	1958
3. Majuro (M)	56.6	+9.8	66.4	-2.7	63.7	-13.4	50.3
5. Bikini (B)	46.4	+13.9	60.3	-2.6	57.7	+7.4	65.1
8. Rongelap (A)	40.8	+38.1	78.9	-14.4	64.5	-3.3	61.2
Average P_1 inbred	47.9	+20.6	68.5	-6.5	62.0	-3.1	58.9
11. A $\times$ M	64.3	+20.3	84.6	-2.8	81.8	-4.7	77.1
12. M $\times$ B	67.8	+7.6	75.4	+4.0	79.4	-8.3	71.1
15. B $\times$ A	70.2	+6.3	76.5	-2.9	73.6	0.0	73.6
Average P_1 crossbred	67.4	+11.4	78.8	-0.5	78.3	-4.4	73.9
Average Difference, P_1 crossbred— P_1 inbred	+19.5		+10.3		+16.3		+15.0
19. AM $\times$ AM	59.8	+11.3	71.1	-7.1	64.0	-6.4	57.6
20. BA $\times$ BA	52.4	+17.9	70.3	-13.3	57.0	-15.0	42.0
21. MB $\times$ MB	62.5	-3.9	58.6	+6.0	64.6	-7.2	57.4
Average F_1 inbred	58.2	+8.5	66.7	-4.8	61.9	-9.6	52.3
25. MB $\times$ BA	84.3	+0.9	85.2	-12.8	72.4	-7.6	64.8
26. AM $\times$ MB	89.4	-5.7	83.7	-5.0	78.7	-6.9	71.8
Average F_1 crossbred	86.9	-2.4	84.5	-8.9	75.6	-7.3	68.3
Average Difference, F_1 crossbred— F_1 inbred	+28.7		+17.8		+14.7		+16.0
Unweighed Average Difference, (P_1 and F_1 crossbred)— (P_1 and F_1 inbred)	24.1		14.1		15.0		15.5

* At the left are given the stocks and their crosses as well as the designations of averages for certain types of crosses. The body of the table gives the per cent egg development each year, the differences between years for crosses and for averages of crosses.

increased viability from 1955 to 1956 (9 of 11 tests) and decreased viability from 1956 to 1957 (9 of 11 tests) and 1957 to 1958 (9 of 11 tests). This might represent environmental effects. It is the common experience for investigators to find that heterozygotes are influenced adversely less than homozygous combinations. On comparing changes in three-way crosses (25 and 26) with this greater stability of the heterozygote in mind, it is seen that there is a small reduction in egg development between 1955 and 1956, and larger reductions between 1956 and 1957, and 1957 and 1958. This would suggest that the laboratory test environment became steadily worse from 1955 to 1958. Figure 5 plots the change in egg development through time using only averages for types of tests. That data in Table 2 are consistent enough in direction of change to make such a comparison seem reasonable, especially since the P_1 crosses and inbreeding were done at the same time, and the F_1 inbred and three-way crosses were done at the same time. Judged by these gross comparisons in Table 2 and Figure 5, there was a greater difference in genetic viability and variability in 1955 than in later years as shown by the greater spread of values and especially by the greater differences in egg development between inbreeding and crossbreeding due to the influence of Bikini and Rongelap genotypes. Also the laboratory environment became worse in 1957 and 1958 although the genetic difference measured between inbred and crossbred tests remained about the same the last three years. We can ascribe the very low values of Bikini and Rongelap in 1955 to the heavy, direct radiation and fallout on Bikini and fallout on Rongelap from the powerful thermonuclear device of March 1, 1954. (No test values have gone down as low as these P_1 inbred in 1955.) The changes other than the recovery of Bikini and Rongelap might also include population cycles in lethal frequencies in these islands which can be demonstrated only by more tests. It should be noted that these two irradiated populations are two of the three strains tested each year. We have already pointed out that the general trend, which also includes tests involving Ponape, is in the same direction.

DISCUSSION

Analyses of the genetic variations in populations are always difficult. Stone *et al* (1957) tried to measure as many variables as possible. In that publication Wheeler and others discussed the ecology and competition within and between species; Spencer showed that the pattern and frequency of visible mutations resembled those analyzed in species such as *Drosophila melanogaster*, *D. simulans*, *D. hydei*, and others; Seecof showed that the cytological variation was conservative, much as in *D. melanogaster*, and that the frequencies and types of rearrangements produced by X-radiation resembled the results obtained with the well analyzed *D. melanogaster* and *D. virilis*. However, Stone and Wilson (1957 and 1958) found that the fertility and especially egg development were inferior to the small or thin desert population of *D. novamexicana* and *D. hydei*.

It is desirable to know about the changes in "genetic load" of detrimental mutants through fluctuations in population size, selection and mutation (including irradiation in the northern Marshalls). This requires repeated sampling over a long period. Some information on the extent of fluctuations that may be ex-

pected can be ascertained in laboratory tests. Gregg (1959, this bulletin) reports on such tests, using two types of population structure: cages, where each cage contained 2000 to 10,000 flies, and pair matings, which consisted of less than 500 pairs. He discusses the techniques and analyses in detail. The only large improvements are from low values toward the general population equilibrium. No test value of egg development was as low as those of the Bikini and Rongelap populations of 1955, either from pair matings or cages. This is in agreement with the data for all four years when the stocks were tested as soon as possible after being brought in from the field. The tests with Ponape crosses show similar relations for 1956, 1957 and 1958. The combined tests given in Table 2 and Figure 5 are made from the two heavily irradiated populations, Bikini and Rongelap, and the control population from Majuro. The greater difference between inbred and crossbred for 1955 is due primarily to the very low viability values of populations from Bikini and Rongelap. The most probable explanation for these low viabilities is that these two populations were still suffering from the increase in number of detrimental mutations from fallout from the March 1, 1954 thermonuclear device. Figure 6 shows a schematic representation of the effect of the thermonuclear device of March 1, 1954 and of subsequent test explosions on the genetic load of adverse mutations in the Bikini populations. Fluctuations in the environment of the islands and in the laboratory tests and the effects of natural selection on the populations prevent our finding such a simple representation of the population in our tests. Adverse environment causes us to overestimate the number of lethals or lethal equivalents, but linkage with the small numbers of autosomes in *ananassae* prevents our detecting the full number of possible adverse combinations. Nevertheless, comparisons of the differences between the results of inbreeding and crossbreeding on the basic factor of evolutionary fitness, viability, allow us to assess and compare the accumulated genetic load of detrimental mutations and gene combinations. Crossbreeding keeps detrimental mutants in the population heterozygous so that only the dominant detrimental component of their action reduces fitness. Inbreeding allows detrimental factors to become homozygous and to occur in a larger fraction of the population as heterozygotes. It is not possible to determine if detrimental effects such as reduction in viability are due to lethals or are the result of a cumulative action of less detrimental factors which in sum are called lethal equivalents. The dominant lethal components of so-called recessive lethals and lethal equivalents (whether heterozygous or homozygous) are often, perhaps always, threshold systems which vary in effect with the remainder of the genotype and the fluctuations of the environment. It is the sum of these factors, good or bad, that determines the evolutionary fitness of a population in a particular environment. We have previously compared (Stone *et al*, 1957; Stone and Wilson, 1958) these natural populations with the extensive studies of Wallace on irradiated laboratory populations, and Gregg (1959, this bulletin) has made further comparisons so we will not repeat those here. Haldane (1957) has recently published an article pointing out the cost of natural selection in replacing a gene in a population by a more effective allele. Our tests do not give us any information on the effect of adding such better alleles that might have occurred in these populations.

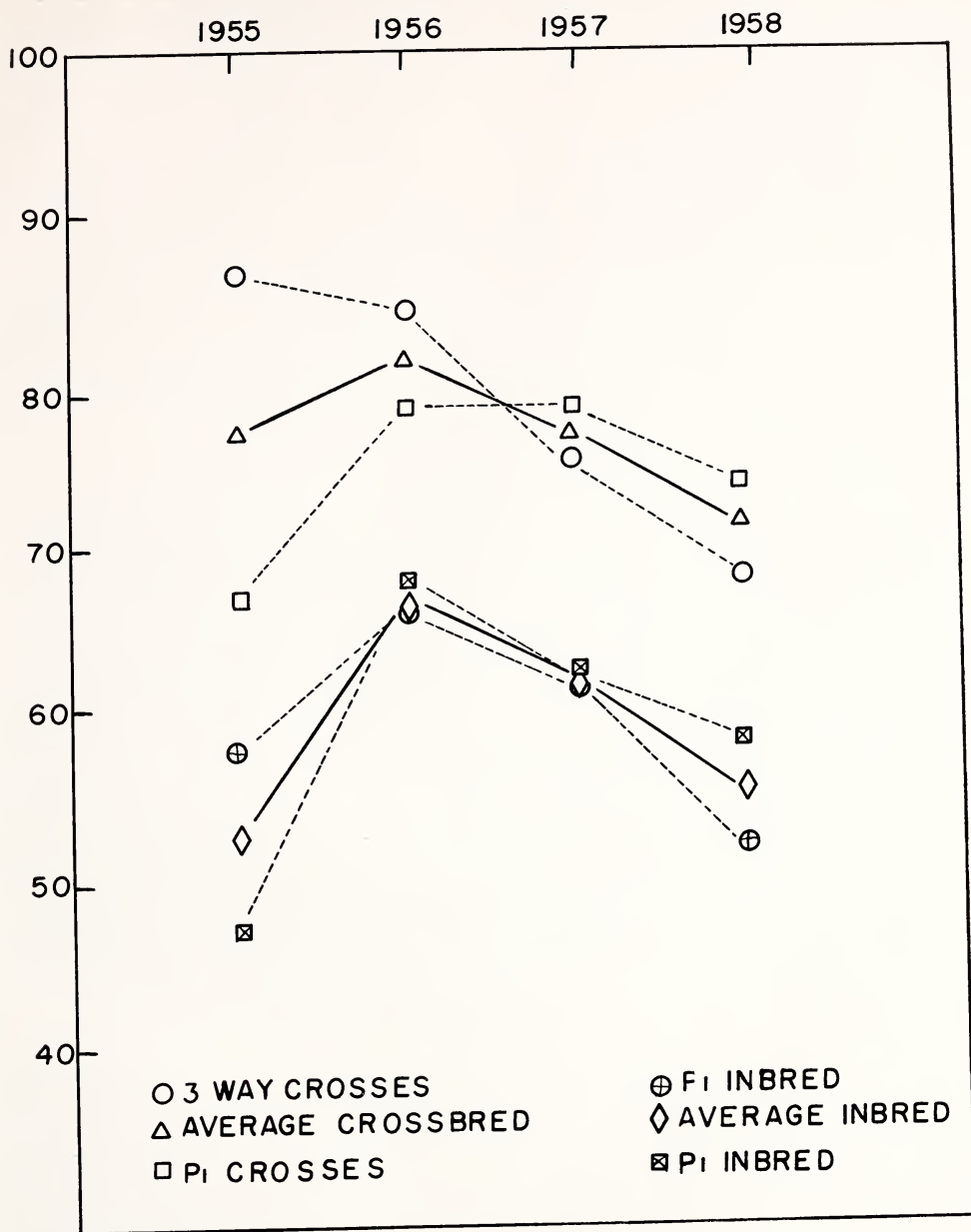


FIG. 5. Comparison of egg development on inbreeding and outbreeding. The difference between the results of inbreeding and outcrossing gives us a measure of the genetic load of detrimental mutations. The plots are for the averages of the tests of the Majuro (control) and Bikini and Rongelap (irradiated) populations each year. In addition the average of P₁ and F₁ inbred and P₁ and F₁ outcrossed is plotted.

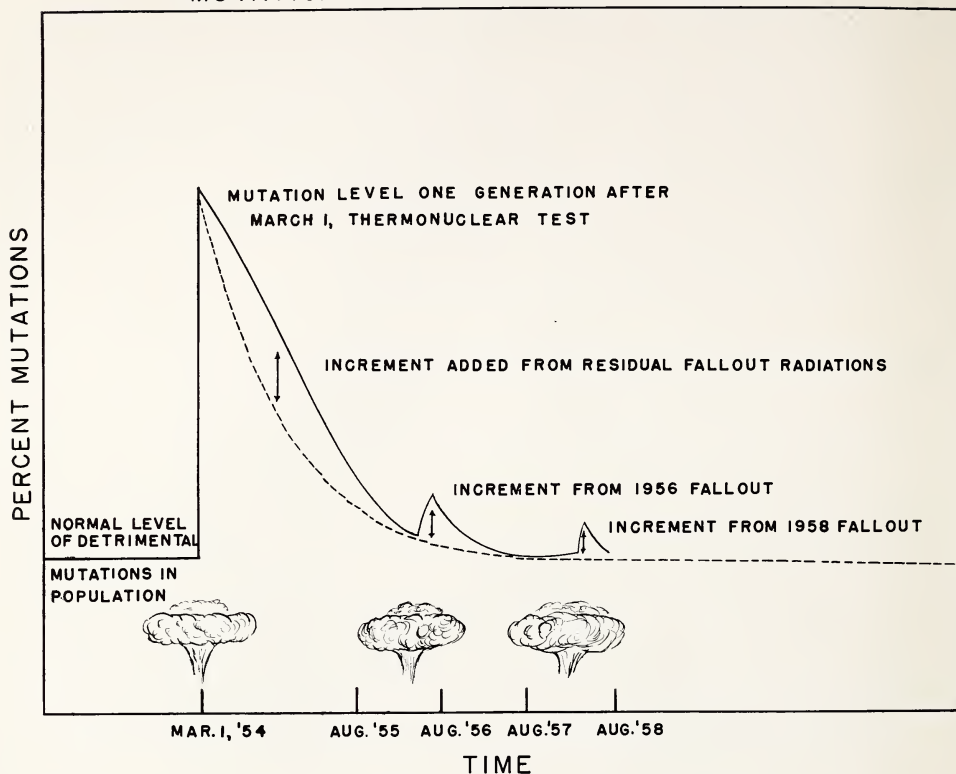
FIGURE 6-EFFECT OF RADIATION ON
MUTATION LEVEL OF BIKINI POPULATION

FIG. 6. Graphic diagram showing the increase in number of detrimental mutations in the Bikini population due to radiations from detonations and fallout and the decay toward a normal level for the population. The Rongelap and Rongerik populations would have a similar but smaller increase in mutations, particularly in 1956 and 1958. With a generation time of 10–15 days, the total number of generations involved between March 1, 1954, and August, 1958, would be about 108–161.

SUMMARY

An attempt has been made to determine the effect of the direct and fallout radiations produced by testing atomic and thermonuclear devices on the genotype of populations of *Drosophila ananassae* living in the area of the Pacific Proving Ground. Collections were made in late July and August in 1955, 1956, 1957 and 1958 from natural populations of *ananassae* on Bikini, Rongelap, Rongerik and Majuro atolls of the Marshall Islands and from Ponape in the eastern Caroline Islands the last three years.

The populations on the island of Bikini received direct and fallout radiations from atomic tests and especially from the thermonuclear device of March 1, 1954. This test contributed a large fraction of the total fallout on Bikini from all tests, and about 99% of the fallout suffered by Rongelap and Rongerik. Majuro and Ponape received very little radiation from the tests so *ananassae* populations from these islands served as control populations. This paper reports the results of test-

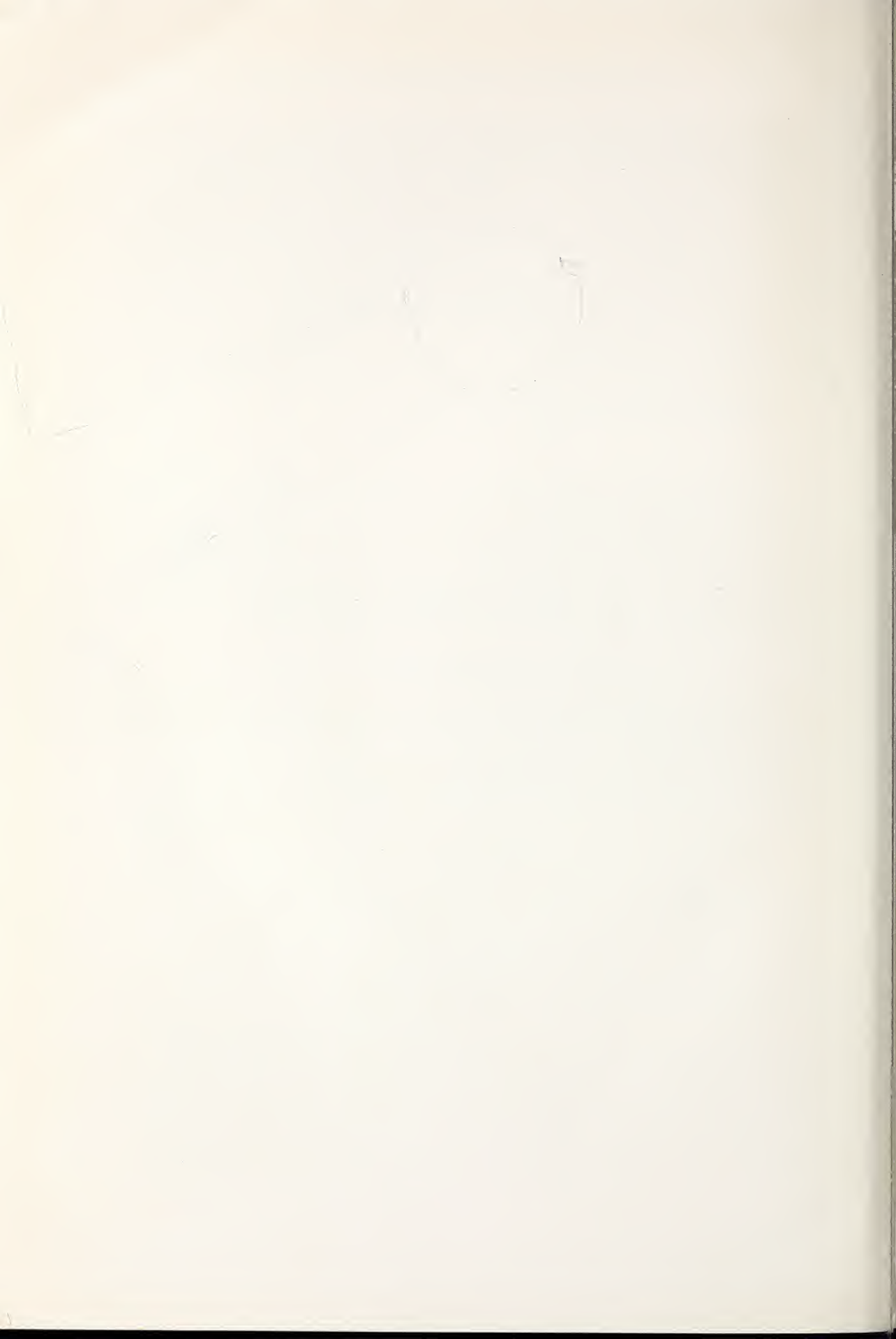
ing the population samples obtained in the summer of 1958. The evolutionary fitness of the populations, measured as fertility, fecundity (egg production per female per day), and especially viability (egg development) was assayed by a series of crossbreeding and inbreeding (brother $\times$ sister matings) tests. The best measure of the genetic load is the comparison between the same genotypes when inbred and crossbred. The genetic load was much greater for Bikini and Rongelap in 1955 (the difference in viability in terms of egg development was greater that year) and has been reduced to about the same level in 1956, 1957 and 1958. This supports the concept that the heavy fallout with its long residual radiation from the March 1, 1954 thermonuclear device had increased the genetic load of detrimental and lethal factors in the two northern Marshall Island populations, but that natural selection had returned these populations to the normal range by 1956 where they remained in 1957 and 1958.

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Some Values of Endomitosis

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In multicellular organisms growth in body size comes about in two ways. Most commonly, of course, there is growth in the size of cells followed by cell division. But in the larvae of many insects, as has long been known, growth in the size of larval organs is due to the enlargement of individual cells which do not undergo mitosis. By larval organs is meant those structures which function in larval life but which undergo lysis when the larva pupates. As we now know, the enlargement of cells in larval organs is due to a real growth and replication of cell organelles without the dissolution of the nuclear envelope, or cytokinesis, a process which is known as endomitosis. After very briefly following the development of the concept of endomitosis the writer wishes to call attention to the adaptive usefulness of this method of cell growth.

When von Mohl, more than a century ago, emphasized that the important part of the cell is the material enclosed within the cell wall—and to which he gave the name of protoplasm—it was soon realized that not everything seen in the protoplasm is living, such as starch grains, oil globules and the like, and this gave rise to much speculation about the nature of the living protoplasm and, indeed, about the nature of life itself. Two general views were widely discussed by biologists during the last quarter of the past century. Many held that the cell is made up of a number of living units, a unique kind of molecule perhaps, or an organized mass of molecules, endowed with the ability to take in and assimilate food materials, to grow, to reproduce and to exhibit all other characteristics of life. The other view was that life is a property of the cell as a whole and is due to the organization and interactions of cell parts or organelles which of themselves are not living.

In his book, *Plasma und Zelle*, published in 1907, M. Heidenhain was one of the last of the older biologists of this era to urge that cells are made up of special and unique living units. Some fifteen years later, a biologist named Jacobj, intrigued, as he wrote, by Heidenhain's arguments sought to put this hypothesis to a test. For if cells are made up of living units capable of self-duplication, then in non-dividing tissue in which there is a wide variation in nuclear and cell size, the volumes of such cells should fall into a geometrical series. The liver of mice was selected for study, first because there is a considerable variation in nuclear size and, second, because normally mitosis does not occur in such tissue. Nuclear diameters were measured and from these nuclear volumes computed. Jacobj (1925) found that in late embryonic life, liver nuclei all had substantially the same volumes and when these were plotted against frequency a single sharply peaked curve was seen. In young adult mice, such a plotting of nuclear volumes gave a double-peaked curve; the first corresponded to the size class found in embryos and the second just double the volume of the latter. Old mice showed three peaks, and their volumes formed a geometrical series, 2 : 4 : 8. etc. From his

studies Jacoby concluded that so far as nuclei are concerned it is evident that a process involving an "*innere Teilung*" of nuclear contents was taking place. At the time, Jacoby's work received little attention but when salivary gland chromosomes came into prominence in the mid-thirties, both Koltzoff and Bridges invoked the concept of "*innere Teilungen*" to explain the large size of salivary chromosomes.

The investigator who gave the first direct morphological evidence about the nature of the "*innere Teilung*" was Geitler (1937) who studied somatic nuclei of larval organs in the water strider, *Gerris lateralis*. In larval tissues of young males, Geitler observed that the single X-chromosome remained condensed, or heteropycnotic, in nuclei. In older males 2 and 4 X-chromosomes appeared in the same type but larger nuclei. This led to a careful study of nuclei of the same size class and Geitler discovered that prior to the appearance of the larger nuclei, all the chromosomes of the nucleus underwent a series of changes closely paralleling the behavior of chromosomes in ordinary mitosis. There was an exact correspondence to the interphase, early, and late prophase in larval organs but the nuclear wall remained intact and no evidence of any spindle could be found. After the chromosomes reached the late prophase, the two chromatids simply separated and the nucleus reverted to the interphase condition. Geitler gave the name "endomitosis" to this method of replicating the contents of a cell. In 1939, Painter and Reindorp showed that endomitosis regularly occurred during the growth in volume of nurse-cells in the ovary of the fruit fly, and this accounted for the great variations in the appearance of the nuclei in this type of cell. Since these early papers, endomitosis has come to be recognized as occurring in all species of plants and animals and this brings up the question, What is the selective advantage of endomitosis over ordinary mitosis so that this method of cell growth is so widely employed in nature? It is this question which has prompted me to prepare this discussion.

The word endomitosis is used for the process in which there is cell growth and the replication of chromosome sets and certain other organelles, without the dissolution of the nuclear wall or the formation of a spindle. As a result cells come to contain many chromosome sets. Thus, in either salivary gland or nurse-cell nuclei, quite commonly as many as 512 or 1024 haploid sets of chromosomes are present. We are not concerned here with the question of how the replicated chromosomes are associated.

In order to understand the adaptive value of endomitosis as a method of cell growth, it is necessary to consider a number of facts, dealing in general with cell chemistry, which have come to light since endomitosis was recognized.

An organism which shows an extremely rapid growth during larval stages is the common fruit fly, *Drosophila melanogaster*, and it is in this species that endomitosis leads to the presence of nuclei which carry high multiples of the original diploid complex. It is very interesting and extremely important to note that the basic problem of larval life is just the same as confronts the organism during the formation of the egg from which the larva arises. In order for an egg to hatch into a larva it must carry within its nucleus and cytoplasm all the food materials needed for embryonic development. Similarly, the larva must assemble in the various tissues of its body all of the materials required for the formation

of the body of the adult fly. It is for this reason that any facts and concepts which shed light of the basic processes involved in oocyte growth are directly applicable to the larval stage as well. Further, since the necessary food materials stored in either the cytoplasm of the egg, or in larval tissues, consist of proteins, nucleic acids and energy-rich compounds of various kinds, any light which can be obtained about the synthesis of such materials is pertinent to a discussion of the question before us.

Since the pioneering work of Caspersson, and Brachet, in the early forties, we have known that the synthesis of proteins requires the presence of ribose nucleic acid (RNA). A vast amount of experimental data, which has accumulated since these early studies, has proved that both DNA and RNA are essential for the synthesis of proteins. For a recent review see Brachet, 1957.

A second well established fact is that large quantities of protein are synthesized in the nucleus of those cells which produce proteins either for storage of such materials in the cytoplasm of a single cell, e.g., oocytes, or for the secretion of such materials by exocrine gland cells. Here it should be noted that part of the protein synthesized in the nucleus appears in the form of nucleoli because Vincent (1955) and others have shown that the main constituent of a nucleolus is protein in a rather concentrated, dehydrated form.

A very remarkable example of protein synthesis within the nucleus is seen in the lateral pharyngeal glands which secrete the royal jelly of the young adult worker bee. Analysis has shown that royal jelly contains about 15 per cent wet weight of proteins. At the time when the adult worker bee emerges from pupation, the royal jelly gland is in an undeveloped state and the gland cells look much like any other diploid cell of the bee. But over the first five or six days of hive life, the cells of the lateral pharyngeal gland undergo four or five endomitotic division cycles so that each nucleus contains from 64 to 128, perhaps more, sets of chromosomes. As the gland cells increase in ploidy the number and size of the nucleoli increases. In an actively secreting gland cell the nucleus is crowded with a high number (up to 80 have been counted in a single nucleus) of large nucleoli each of which is rich in RNA. As the gland cell ceases to secrete the royal jelly after some eleven days of hive life, the nucleoli shrink in size, lose their RNA and finally disappear completely.

Another very illuminating fact has recently been established by Allfrey and Mirsky (1958) who have shown not only that DNA is essential for the production of protein within a nucleus but also that the amount of protein a nucleus can synthesize depends on the amount of DNA it contains. These authors studied nuclei of mammalian thymocytes, isolated in a sucrose solution. When all of the essential amino acids are added to the medium, by the use of radioactive isotopes they have shown that protein is synthesized by these nuclei. If the DNA of nuclei is slowly removed by the use of DNase, in measure, the ability to synthesize proteins falls off. Nuclei depleted of their DNA can regain some of the ability to synthesize proteins if DNA from any source is made available to them. Most recently, it has been shown that a polyanion, which is not structurally related to the nucleic acids, can be substituted for DNA. From this it appears that, in part at least, the DNA has a non-specific effect in protein synthesis and functions because of the configuration of its constituent molecules.

From the work of Allfrey and Mirsky the conclusion may be drawn that the ability of any diploid nucleus to synthesize protein, other things being equal, depends to a considerable extent on how much DNA it contains, or more probably on the amount of a special kind of DNA it contains. It is tempting to suggest that this special sort of DNA is carried by heterochromatic regions but as yet there is no direct evidence of this. However, the chemical heterogeneity of DNA is now a well established fact. As (diploid) sets of chromosomes are increased within a nucleus, by endomitosis or other means, the capacity of such a nucleus to synthesize proteins is greatly increased.

One more factor must be considered; this is the topographical relation between exocrine gland cells and the source of precursors for secretory products. Whether one considers animals with a closed circulatory system, or an open one, the essential fact is that the base of the gland cell is directly exposed to a source of food. In the pancreas of man, for example, the base of the gland cell lies adjacent to a capillary network in the basement membrane, or in the fruit fly the salivary gland lies freely in the body cavity and is directly exposed to the haemolymph. The distal surface of the gland cell usually forms the gland duct.

The adaptative value of endomitosis is well illustrated by the salivary gland of *Drosophila melanogaster*. At the time of hatching, the salivary gland contains around 130 cells and since, on the average, some four cells abut on the duct, at any given level, the gland is about 30 cells long. As larvae grow, the need for more digestive enzymes can be met in one of two ways. The number of secretory cells might be increased by mitosis. The alternative would be for the synthetic apparatus of the gland cell to be increased without cell division.

Because of the topographical relation between a gland cell and a source of food, it is readily apparent that to increase the number of cells would produce a gland of great length. For example, the length of the salivary gland of the fruit fly, at the time of hatching, is about 0.3 millimeters. If all gland cells were to undergo a single mitotic division, the length of the gland would about double. It is well known, of course, both from cytological analyses of the chromosomes, nuclear volumes, and other evidence, that salivary chromosomes contain up to some 512 to 1024 chromatids which are the product of 6 to 8 endomitotic division cycles. If the length of the salivary gland were doubled even 6 times, the gland would be 19 millimeters long which would be extremely difficult to house in a mature larva with a body length of only 6 to 8 millimeters. In addition the problem of resistance to flow of secretion products would be very great in a long thin gland.

Endomitosis allows for a growth of cell volume in three dimensions and at the same time preserves the topographical relation needed for secretion. When the volume of a cell is doubled each of the three dimensions is not so greatly affected. Embryological studies indicate that during larval development there is actually about a 20-fold increase in the length of the salivary gland and presumably similar increases in the other two dimensions.

In addition to the problems introduced by excessive gland length, from the standpoint of metabolism and synthetic activities mitosis would entail a loss of much time. In the case of an amoeba, for example, which normally divides once in 24 hours, Mazia (1955) has shown that synthetic activities go on for about

18 hours and the rest of the daily cycle is concerned with the separation of replicated cell parts. Presumably, similar time relations exist in other dividing cells. During endomitosis there is no need for a cessation of secretory activity because the replication of chromosomes, and other cell elements normally takes place in early interphase, a time when other synthetic processes are at a high level.

From the evidence which has been presented it is abundantly clear that endomitosis has great adaptive value to an organism in which true growth is restricted to a relatively short period of the entire life cycle, or in other situations which call for the rapid synthesis of proteins, nucleic acid precursors and other materials to meet the special needs of an organism. In the fruit fly true growth from food outside the organism (gonads excepted) is confined to the larval period which ordinarily lasts about 96 hours. The continuous ingestion of food over this period requires the continuous production of digestive enzymes to convert such food into a form available for assimilation by the soma cells. The replication of chromosomes increases the capacity of the nucleus to synthesize nuclear proteins and an increase in cytoplasmic volume not only increases the basal area of gland cells, for the intake of food, but also adds to the capacity of cytoplasmic organelles which synthesize proteins from such food.

Examples of cases in which large quantities of proteins and other materials are required for special purposes are widespread in nature. Notable are nurse-cell mechanisms so prominent in the ovaries of invertebrates and especially insects. In order for a zygote to function effectively it must contain within its nucleus and cytoplasm all the proteins, nucleic acid precursors and energy-rich compounds required for the development of the embryo to the stage when it can obtain food from its environment. In the fruit fly this function of reserve food synthesis is largely delegated to specialized nurse-cells which serve as unit factories for the production of essential materials. As was shown some years ago (Painter and Reindorp, *loc. cit.*) the nurse-cells of *Drosophila* undergo endomitosis and reach a degree of ploidy comparable to that of salivary gland cells. Eventually, the contents of nurse-cells are incorporated into the cytoplasm of the oocyte. Throughout the whole animal kingdom, it is the rule for young oocytes to be intimately associated with one or more nutritive cells.

Another example in which endopolyploidy plays a very important role is in the lateral pharyngeal glands of young adult worker bees. As I showed in an earlier paper (Painter, 1945) each of the cells which secrete the royal jelly, during the development of this gland, have the synthetic capacity of the gland cells increased in two ways. Initially the young diploid gland cell is associated with a nutritive nurse-cell which is at least tetraploid in chromosome constitution. Eventually the nurse-cell is engulfed by the gland cell and after this there are at least four endoreplications of the chromosomes. The high number and the large size of nucleoli, in actively secreting cells, attest to the fact that nuclear proteins are being synthesized in large amounts.

In conclusion, the writer feels that in spite of the fact that our understanding of cell chemistry is still very fragmentary, many cytological phenomena, long known from the morphological standpoint, can now be profitably considered again in the light of our present knowledge and in this sense an understanding

of the adaptive values of endomitosis in the economy of organisms opens the way for other such explorations.

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Alexander von Humboldt and the Science of the Nineteenth Century¹

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I

The year of the death of Alexander von Humboldt (1859) marks a milestone in the History of Science. The same year appeared the first edition of Darwin's *Origin of Species* and with it, started by the evolution theory, there developed a completely new Biology. That was the origin of those very revolutionary trends of our twentieth century science which obtained their most obvious shape during the first years of our century with Planck's Quantumphysics and Mendel's—*sit venia verbo!*—"Quantum" Genetics. By these absolutely new scientific creations the so-called classical physics of Newton, with Helmholtz and Lord Kelvin as its last significant representatives, and classical biology from Linné to Cuvier, have been definitely brought to their final historical perfection. And it is to be the principal task of this essay to show that Alexander von Humboldt's scientific work has given us a most perfect and splendid synthesis of that eighteenth and nineteenth century science which today we call "classical."

Such a change, not only in centuries but in eras, comprehends not only changing ideas, principles and ideals in science, but also practical methods of gaining scientific facts and experience such as voyages of exploration, for instance. Humboldt is a very good example for this, also. His great American voyage illuminates in the same way the character and spirit of the classical epoch of modern science, as do also the leading philosophical ideas of that time. The most noble way to exercise science in Humboldt's time was to make exploring expeditions and research voyages; in our own twentieth century it is the research *Institute*. Research voyages are, of course, being made today, but we are making them, so to say, in the form of travelling research institutes. To understand clearly the essential difference between research travelling during the nineteenth century and our own modern method, we have to compare only the arctic expedition of Nansen with the antarctic expedition of Admiral Byrd. The polar expeditions of the latter twentieth century have been steadily-growing research institutes, travelling by boat, motor-sled and plane; Nansen travelled by dogsled and by skiing. It is hard for us to imagine that Nansen, only some few thousand miles away from his home country, Norway, was without any communication with it for more than four years; Nansen was realizing his research expedition in much

¹ A Humboldt-Commemoration lecture delivered in April, 1959, at the University of Texas, Austin, at the American Academy of Sciences and Arts, Boston, and at the University of Toronto, Canada. The footnote translations of German quotations were made by Prof. Silber of the University of Texas to whom the author expresses his great indebtedness.

the same way that Humboldt did it a century ago, whereas Admiral Byrd, only a few years later, applied all of the great achievements of our technocracy. So, with the changing century, the age of individual and personal scientific travelling is also definitely gone, and the era of liberalism and personalism in science has been replaced by the still steadily-growing teamwork plan of today.

Just as Humboldt's work during his epoch was outstanding, so also was his great American voyage in the history of research voyages. Before him and after him there have been a great many scientific travellers of his kind, but none of them has left behind the same glorious memory of his doings as did Humboldt. The many mountains, rivers, streams, cities, counties and other places which bear his name record it. There has to be a reason for this, which we will have to consider first in order that we may understand better Humboldt's work. The principal reason, it seems to me, has been the fact that Humboldt's entire work—his practical research voyage as well as his theoretical books—are guided by one and the same philosophical and universal idea which he himself described as "*l'idée d'une physique du monde*" (in a letter to his friend Pictet) or later by the single word "*Kosmos*" which we may now characterize as the idea of a philosophy of the earth or of Geography. The second reason for Humboldt's unique success has been the historical fact that he represented the very end of his epoch, so that he was able to summarize and synthesize the immense amount of ideas, facts, and ideals that the ending of any era has to offer. And, finally, the last reason for his splendid results has been the very charming personality of Humboldt himself, being a nobleman of the cosmopolitan education of Goethe's time and also being financially completely independent. All of these characters came together to shape Humboldt's work and his personality into the outstanding and surpassing figure of classical modern science that we know.

We must examine this in all of its details, for in doing so we are not only fulfilling a request of the past history of science but we are also serving the immediate philosophical necessities of our present time. To clarify that, I will use a somewhat risky allegory, which compares the history of science with political history. Machiavelli stated somewhere that in political history the neighbor of any state is always its enemy but the neighbor of the neighbor is its friend. Just the same has always been true for the sequence of historical epochs and generations. For any present time has as its antagonistic opponent its immediate predecessor, whereas the predecessor of the predecessor always has the meaning of a congenial collaborator for it. In this way an examination of Humboldt's time, as the antagonist of its own successor and as our own predecessor, will, I hope, help us in clarifying the darkness of the spiritual horizon before us.

II

What distinguishes Humboldt's work fundamentally from all similar undertakings of his contemporaries is, as I said above, the fact that not only his whole theoretical but also his empirical and experimental research was guided by one and the same universal idea which served him constantly as his philosophical ideal of knowledge. Humboldt himself defined it as "*l'idée d'une physique du monde*" or, in one word, as the idea of the "*Kosmos*." This philosophy of the earth

or geography accompanied him throughout all his life. Before beginning his great American voyage he wrote from Madrid in a farewell letter to his friend Baron von Moll concerning his main intentions on that exploration voyage: "*auf das Zusammenwirken der Kräfte, den Einfluss der unbelebten Schöpfung auf die belebte Tier- und Pflanzenwelt, auf diese Harmonie sollen stets meine Augen gerichtet sein.*"² And immediately after his return from that long trip he defined anew the general task of his voyage and his literary work in his first general and most popular book, "*Ansichten der Natur*" (Views or Aspects of Nature): "*Ueberblick der Natur im Grossen, Beweis von dem Zusammenwirken der Kräfte, Erneuerung des Genusses, welchen die unmittelbare Ansicht der Tropenländer dem fühlenden Menschen gewährt,*"³ that is the main purpose of this book. At the end of his life, in "*Kosmos, Entwurf einer physischen Weltbeschreibung*" (Cosmos, a General Survey of the Physical Phenomena of the Universe), the "book of all his life" as he himself tells us, he states for the last time: "*Das Grundprinzip dieses Werkes ist in dem Streben enthalten, die Welterscheinungen als ein Naturganzes aufzufassen.*" So the book will give us "*die denkende Betrachtung der durch Empirie gegebenen Erscheinungen als eines Naturganzes.*"⁴ With good reason one of his biographers (J. V. Carus in Bruhns 1872) states that Humboldt was always more deeply interested in the mutual correlation of the facts than only to know isolated facts.

This universal philosophical idea to deliver a whole "philosophy of the earth" comprehends not only the work which Humboldt himself accomplished, it marks too the still-living tradition of it throughout the history of science which is again becoming strong in our own time. Our discourse, then, has now a double task to fulfill; first it has to present a sketch showing how Humboldt has realized it by his own work, and then we must characterize the trends of his main ideas and ideals of knowledge which continue to work as a living tradition in our actual situation of science.

The first and indeed immensely great undertaking to realize this "cosmic" philosophy was Humboldt's great American voyage, or his "*Voyage aux régions équinoxiales du Nouveau Continent, fait en 1799, 1800, 1801, 1802, 1803 et 1804,*" as it is given in the title to his great opus concerning this voyage, consisting of thirty large volumes and which appeared in Paris from 1808-1834. This gigantic work represents a real *opus scientificum iberoamericanum*, and marks the start of modern scientific research in Latin America. Official representatives of the University of Habana, Cuba, conferred sometime a mark of honor upon the monument of Humboldt which stands before the University of Berlin, which names Humboldt the second discoverer of Cuba (after Columbus). It seems to me that we are not really saying too much to characterize Humboldt as the scientific discoverer of Latin America. His *opus americanum* encompasses

² "upon the coordination of forces, the influence of inanimate creation on the animate world of plants and animals, upon this harmony shall my eyes be constantly directed."

³ "The survey of gross nature, the demonstration of the coordination of forces, the renewal of enjoyments which the immediate inspection of tropical lands gives to sensitive men."

⁴ "The basic principle of this work is expressed in the struggle to comprehend the appearances of the world as a totality of nature." So the book will give us "the thoughtful contemplation of the whole of nature as given empirically through appearances."

all of the sciences of his time: Biology, Geography, Geology, Geophysics, Zoology, Archaeology, History, etc., and furthermore, he has created a field of science which did not exist until this time, the Geography of Plants. Also it is true that his grandiose geographical monographs, particularly those dealing with Mexico and Cuba, have for the first time made Geography as a real and exact science. Before Humboldt Geography was little more than a collection of more or less interesting facts or curiosities, and could scarcely be called a science. For Humboldt himself, the immense collections of facts and experiences had meaning only in providing him with all that empirical experience which is needed to obtain a philosophy. But the creation of such a philosophy needs a man whose eyes are looking not only at the heavens but whose feet are also strongly rooted in the soil because, as Humboldt says (Bruhns 1872): "*Man schadet der Erweiterung der Wissenschaft, wenn man sich zu allgemeinen Ideen erheben und doch die einzelnen Tatsachen nicht kennen lernen will.*"⁵ In the aforementioned *Ansichten der Natur*, his most favorite book as well as that of the general public, we have the best illustration of how he connected ideas with facts. This book consists of a series of independent essays of high literary rank written in the most noble German created by Goethe, Humboldt's best friend and, as we later will recognize, also his best adviser in science. Each of these essays represents in itself a "microcosmos" about its special theme (as compared with the great "Kosmos" comprehending the universe as a whole) and each is accompanied by a long series of notes which give references to all the facts concerned, correlating them intimately with the ideas in the corresponding essays. Many of these notes represent in themselves small scientific treatises based upon observations and exact measurements and often consist of many times more pages than the essays themselves.

But it is not only bibliographically—in the logical structure of his books—that we recognize that Humboldt's collecting of facts and experience was always guided by his philosophical ideas; we also have the good testimony of the voyage itself, testimony which reveals to us at once also the spiritual origin of his cosmic idea. Humboldt writes (in *Ansichten der Natur*): "*In den Wäldern des Amazonenflusses wie auf dem Rücken der hohen Anden erkannte ich, wie von einem Hauch beseelt von Pol zu Pol nur ein Leben ausgegossen ist in Steinen, Pflanzen und Tieren und in des Menschen schwellender Brust. Ueberall ward ich von dem Gefühl durchdrungen, wie mächtig jene Verhältnisse in Jena auf mich gewirkt, wie ich durch Goethes Natureinsichten gehoben, gleichsam mit neuen Organen ausgerüstet worden war.*"⁶ Here we are at the real origin of Humboldt's cosmic ideas and philosophy. They are most intimately connected with Goethe's natural science and philosophy. Here, too, we are at the starting point to prove and substantiate the main thesis of our lecture—that Humboldt represents the culmination point and the highest perfection of the natural science of Goethe's time. Only

⁵ "Now the expansion of science suffers if one rises to universal ideas and yet is not willing to learn the individual facts."

⁶ "In the forests of the Amazon River as upon the ridges of the high Andes I recognized how only *one* life, animated by *one* breath from pole to pole, is diffused in stones, plants, and animals, and in the swelling breast of man. Above all, I was filled with the feeling of how powerfully these relationships affect me in Jena, of how I had been elevated and, at the same time, equipped with new organs by Goethe's *Insights into Nature*."

in connection with it do Humboldt's results and ideas show their real sense and high value.

III

The last five years of the eighteenth century Humboldt spent preparing spiritually and practically his great American voyage which started June 5th, 1799, in La Coruña, Spain. Essential for these years were Humboldt's various visits with Goethe in Jena and Weimar during the years 1794-97 and the following visit in Paris where at this time the best scientists of Europe worked together at the Paris Academy and Natural History Museum. In the circle around Goethe, Humboldt conceived and evolved his natural philosophy and the general principles of his scientific thinking, whereas in Paris he learned the practical methods to establish by modern exact measuring scientifically assured facts and experience. Upon these fundamental principles are especially based the *original* scientific creations of Humboldt's—his morphological geography of plants and his physiology.

Goethe, as is well known, was a morphologist in all his science, even in Anatomy and Botany just as in his doctrine of colors and his essays about philology and history. The word "morphology" itself was created by Goethe. The basic principles of Goethe's, the so-called "idealistic morphology" (Naef 1919) have also been the fundamental principles upon which Humboldt based his plant geography and all his philosophy of the Kosmos. And we are able to determine exactly the meeting point between Goethe's and Humboldt's thinking: it is fixed in the first personal meeting of them during the year 1794. In his corresponding diary Goethe writes about this meeting: "*Alexander von Humboldt, längst erwartet, von Bayreuth ankommend, nötigte uns ins Allgemeinere der Naturwissenschaft . . .*" And in his "*Nachträgen zur Osteologie*" Goethe points out more precisely about the matters they have been discussing, namely: "*Ich trug die Angelegenheiten meines Typus so oft und zudringlich vor, dass man, beinahe ungeduldig, zuletzt verlangte, ich solle das in Schriften verfassen, was mir im Geiste, Sinn und Gedächtnis so lebendig vorschwebte.*"⁷ Following this proposal Goethe wrote, in the year 1795, therefore immediately after Humboldt's visit, his famous "*Erster Entwurf einer allgemeinen Einleitung in die vergleichende Anatomie, ausgehend von der Osteologie,*"⁸ which I consider to be the best and most thorough treatise of all the biological writings of Goethe. Here he evolves his three fundamental principles of Morphology, which—no accident, of course—are just the same principles upon which Humboldt based his new science of Plant Geography.

To show that, we must first evolve Goethe's views of these principles. They are three: the principle of *type* in morphology, the *compensation* principle, and the

⁷ "Alexander von Humboldt, long awaited, arriving from Bayreuth, forced us into the universal aspects of natural science . . ." And in his *Supplements to Osteology* Goethe points out more precisely about the matters they have been discussing, namely: "I expressed this business of my *Type* so often and so obtrusively that one finally demanded, almost impatiently, that I set down in writing that which hovered so vitally before my mind, sense and thought."

⁸ "First proposal of a General Introduction to Comparative Anatomy, derived from Osteology."

principle of *holism* (as it is called today, after Smuts, to underline its antagonism to the principle of mechanism). The principle of *type* used by Goethe and Humboldt has nothing to do with the phylogenetic types of our present Biology based upon the Evolution Theory. Goethe's type represents a pure ideal construction without any historical meaning, wherefore Naef has named this kind of morphology "idealistic morphology." Indeed, this is all really true Platonism. Goethe very often confessed himself to be a Platonist: "*Um sich aus der grenzenlosen Vielfachheit, Zerstückelung und Verwicklung der modernen Naturlehre wieder ins Einfache zu retten, muss man sich immer die Frage vorlegen: wie würde sich Plato gegen die Natur, wie sie uns jetzt in ihrer grösseren Mannigfaltigkeit, bei aller gründlichen Einheit, erscheinen mag, benommen haben?*"⁹ Unity in the multiplicity—that is indeed the most concise formula to define the platonic idea, and it serves equally well to define the type in Goethe's idealistic morphology. The ideal geometrical circle, for example, represents the ideal unity in the multiplicity of an infinity of real circles, each one of them somewhat different from all the others. And just the same is true of the relation of the ideal type of any morphological species of plant or animal to the almost infinite multiplicity of the really existing individuals of those species, each of them somewhat different from all the others. So any morphological type, too, always represents the ideal unity of a real multiplicity.

We recognize that immediately, if we hear now how Goethe himself defines his anatomical type in the above-mentioned treatise: "*Deshalb geschieht hier ein Vorschlag zu einem anatomischen Typus, zu einem allgemeinen Bilde, worin die Gestalten sämtlicher Tiere, der Möglichkeit nach, enthalten wären, und wonach man jedes Tier in einer gewissen Ordnung beschriebe. Dieser Typus müsste soviel wie möglich in physiologischer Rücksicht aufgestellt sein. Schon aus der allgemeinen Idee eines Typus folgt, dass kein einzelnes Tier als ein solcher Vergleichskanon aufgestellt werden könne; kein Einzelnes kann Muster des Ganzen sein. . . . Die Idee muss über dem Ganzen walten und auf eine genetische Weise das allgemeine Bild abziehen.*"¹⁰ Remember that the term "genetic" here means typological-genetic but not phylogenetic. Take, for example, Goethe's "metamorphosis" of the leaf into the flower, which illustrates exactly the typological genetic development but of course not a real historical phylogenetic evolution. The best samples for Goethe's ideal types are his "*Urpflanze*" (original plant) and his "*Urtier*" (original animal). They represent for him ideal anatomical models for possible physiological construction of new kinds of really possible plants and animals. In a letter written in 1787 from Rome to Mrs. von

⁹ "In order to preserve oneself in simplicity from the limitless diversity, partitioning and complication of the modern theory of nature, one always must pose to himself this question: How would Plato have maintained the basic unity of nature had he confronted her, as she now appears to us, in far greater multiplicity."

¹⁰ "A proposal, therefore, is put forward here of an *anatomical type*, of a universal picture, wherein the forms of all animals, according to their possibility, would be included. And according to this type, or picture, each animal can be described in a certain order. This type must be based, so far as possible, on physiological considerations. From the universal idea of a type it follows that no single animal could be proposed as a paradigm for comparison. No individual can be a model for the whole . . . The idea must rule over the totality and, according to a genetic method, derive the universal picture."

Stein, Goethe says from his *Urpflanze*: "Mit dem Modell und dem Schlüssel dazu kann man alsdann noch Pflanzen ins Unendliche erfinden, die konsequent sein müssen, d.h., die, wenn sie auch nicht existieren, doch existieren könnten und nicht etwa malerische oder dichterische Schatten oder Scheine sind, sondern eine innerliche Wahrheit und Notwendigkeit haben. Dasselbe Gesetz wird sich auf alles übrige Lebendige anwenden lassen."¹¹ In this sense, as typological-physiological models but not as real historical beings, Goethe understood his *Urpflanze* and *Urtier* as anatomical types, and as we will see, this is true also of Humboldt's "*Physiognomische Grundgestalten*" (physiognomic groundforms) in his plant geography.

The second typological-morphological fundamental principle from Goethe which Humboldt also applied in his plant geography is the so-called *compensation principle*. It defines the basic postulate which any typological transformation—named metamorphosis by Goethe—has to fulfill if it is a possible one and a "true" one. Goethe says this about it: "*Betrachten wir nach jenem, erst im allgemeinen aufgestellten Typus die verschiedenen Tiere der vollkommensten, die wir Säugetiere nennen, so finden wir, dass der Bildungskreis der Natur zwar eingeschränkt ist, dabei jedoch, wegen der Menge der Teile und wegen der vielfachen Modifikabilität, die Veränderungen der Gestalt ins Unendliche möglich werden. ... Wenn wir die Teile genau kennen und betrachten, so werden wir finden, dass die Mannigfaltigkeit der Gestalt daher entspringt, dass diesem oder jenem Teil ein Uebergewicht über die andern zugestanden ist. ... So sind, zum Beispiel, Hals und Extremitäten auf Kosten des Körpers bei der Giraffe begünstigt, dahingegen beim Maulwurf das Umgekehrte stattfindet. ... Bei dieser Betrachtung tritt uns nun gleich das Gesetz entgegen: dass keinem Teil etwas zugelegt werden könne, ohne dass einem andern dagegen etwas abgezogen werde, und umgekehrt. ... Der Bildungstrieb ist hier in einem zwar beschränkten, aber doch wohl eingerichteten Reiche zum Beherrscher gesetzt. Die Rubriken seines Etats, in welche sein Aufwand zu verteilen ist, sind ihm vorgeschrieben; was er auf jedes wenden will, steht ihm, bis auf einen gewissen Grad, frei. Will er der einen mehr zuwenden, so ist er nicht ganz gehindert, allein er ist genötigt, an einer andern sogleich etwas fehlen zu lassen; und so kann die Natur sich niemals verschulden oder wohl gar bankrott werden.*"¹² Both of these fundamental prin-

¹¹ "With this model and with the key thereto one can discover an endless number of plants which, as a consequence, must be. That is to say, these plants, even if they do not exist, could nevertheless exist. They are not some pictorial or poetic images or appearances; rather they have an inner truth and necessity. The same law can be applied to all other living beings."

¹² "If, after that, we consider first the most perfect in the most general established types of different animals, which we call mammals, we find that the organizational system of nature is indeed limited. Nevertheless, because of the multitude of parts and because of the diversity of capacities for modification, the alteration of forms *ad infinitum* becomes possible. . . . If we know and consider the parts exactly, we will find that the multiplicity of form springs from the fact that this or that part is granted the upper hand over the other. . . . So, for example, in the giraffe the neck and extremities are favored at the expense of the body. The reverse situation is found in the mole. . . . By means of this consideration we advance at the same time toward the law: that no part will be able to increase unless something is, on the other hand, taken from another part, and vice versa. . . . The organizational impulse here is put forth as the governor of a Kingdom, indeed limited but nonetheless well directed. The rubrics of its budget in which

ciples of the typological morphology from Goethe and Humboldt are based upon one and the same still more fundamental biological principle, that same one which overran and exceeded Humboldt's own epoch, helping us today to overcome the mechanistic philosophy of nature of the second half of the last century. This principle we call today the principle of *holism*, after Smuts, and which, of course, is antagonistic to any mechanism. Speaking here of mechanism and holism we do not mean the mechanistic or holistic general philosophy of nature, but both principles as *causal* ones which are helping us to form exactly demonstrated scientific theories. So, for example, when we are speaking of the mechanism of atomic activity or of respiration, then we may say that we are able to explain these complicated phenomena in the causal way of exact physical or physiological research. In this sense mechanism and holism as *causal* principles are antagonistic and opposed principles but they are not contradictory in the sense of logic. There are many phenomena in nature that we are able to explain as pure mechanisms, but there are also others that we may understand only as holisms. So the causal principles of mechanism and holism are not contradictory ones, as the corresponding metaphysical systems of mechanism and holism are, because they—as all philosophical systems are—are concerned always and principally with *totalities* of reality, whereas scientific theories are just as fundamentally concentrated in *particular realities*.

The holistic causality we cannot define better than was done by Christian von Ehrenfels by his statement that "the whole represents more than just the sum of its parts." If we are not able to explain a complicated phenomenon of physical or biological nature by mechanistic causality, which means to analyze it into its most simple elements and then to summarize it out of these simple elements, then we are dealing with a phenomenon which is to be understood only in the way that holistic causality points out. That means that we have to try to explain the activities of the parts of any whole or holism only by starting with the whole as such, which of course then has to be known to us. This is precisely the case in Goethe's and Humboldt's morphological types. The possible compensations in and by them we are able to consider and construct only if first we have a clear idea of our type as a whole or as an holism in the sense of Smuts. Whereas the strongly mechanistical epoch, which followed the Humboldt-Goethe-Time and preceded our present time, has been absolutely convinced that in some future time we would be able to explain all phenomena of nature, including the most complicated biological and psychological ones, by the way of mechanistic causality, today we recognize more and more phenomena as absolutely not being able to be explained sufficiently as pure mechanisms. And this is true not only in psychology and biology but in microphysics too. From Planck himself, we can understand a microphysical material-wave only if we consider it as a whole, the behavior of its microelements, quanta, being determined only by the wave as a holism, and not, reciprocally, the behavior of the wave as a whole summarized by the singular behavior of its microconstituents. And the so-called Pauli-prohibi-

its expenditures are to be divided, are preordained. What it wishes to spend on each is, to a certain degree, uncontrolled. If it wishes to spend more on one it is not completely prevented from doing so. It is merely required, at the same time, to leave something lacking in another. And so nature can never go in debt nor become completely bankrupt."

tion, a fundamental postulate of modern atomphysics, has been recently characterized by March as a typological-morphological principle—in the sense of Goethe and Humboldt—and not as a principle of causal physics. It is indeed, biologically considered, really a morphological axiom of the same observance as the compensation principle of Goethe, to which group of logical principles it belongs.

Our statements have made it perfectly clear that neither Humboldt nor Goethe could be mechanists. As for Humboldt, we will recognize that subsequently also when dealing with his physiology. There we will see that his use of the term "*Lebenskraft*" (vital force) does not mean that he was a vitalist. Not at all! It means only that he represented modern holism in the then usual Goethe-Schelling fashion and, of course, with an obviously anti-mechanistic tendency. Now we have to show how Humboldt applied the mentioned principles of Goethe's typological morphology in his *Plant Geography*. This science did not exist before Humboldt; it represents his own and his most original scientific creation which endures his particular epoch and ideas, being a new science of its own independent historical existence. It has been said that all Humboldt accomplished has been geography, or at least more or less intimately connected with geography. With the exception of his physiology, that is absolutely true, and we may add that his geography culminated in his *Plant Geography*. Here we find together all that is true and perfect in Humboldt's work. The corresponding book is titled: "*Essai sur la géographie des plantes; accompagné d'un tableau physique des régions équinoxiales... Paris 1805.*" In the 30-volume *Corpus Americanum* it bears the number XXVII, but it was the first book which appeared of the whole series. Humboldt himself translated it into the German language, the German edition being titled: "*Ideen zur Geographie der Pflanzen, nebst einem Naturgemälde der Tropenländer, auf Beobachtungen und Messungen gegründet, welche vom. 10. Grade nördl. bis zum 10. Grade südl. Breite in den Jahren 1799–1803 angestellt worden sind. Tübingen 1807*", 4°, XII, 182 p. with 1 Tab. It has been dedicated to Goethe who has been said to have devoured it, and he was so enthusiastic about it that he immediately painted the corresponding table, which was still lacking in his first specimen of the book. If now we have to characterize the kind of plant geography Humboldt has realized here, we can do it with one word only: it is a very significant *typological* plant geography, typological in precisely the sense that Goethe used the term.

Today plant geography, like all other biological sciences, is based absolutely upon the evolution theory. That could not be the case with Humboldt, whose book appeared more than half a century before Darwin. All his statements about the matter are clearly correlated with the principles of Linné and Cuvier concerning the constancy of species and types. Therefore all historical transformations and changes in the composition of the vegetation on earth are only due to the geological cataclysms and subsequent new immigrations from neighboring territories, as Cuvier has instructed us. Says Humboldt: "*Die urtiefte Kraft der Organisation fesselt, trotz einer gewissen Freiwilligkeit im abnormen Entfalten einzelner Teile, alle tierische und pflanzliche Gestaltung an feste,*

ewig wiederkehrende Typen."¹³ Are those not almost the same words Goethe used to describe his "general type", which we quoted above? But we have to distinguish between two principally different kinds of types, the homologue-ones, which constitute the taxonomical "Natural System" of organisms and which are connected particularly with the characters of the sexual organs, the so-called organization-characters, and the analogue-ones, which refer especially to the functional similarities or so-called convergences, and which are connected particularly with the vegetative organs of the organisms. You remember the interesting historical fact that Linné gained his overwhelming success in forming his plant-system by using the organization-characters of the sexual systems, whereas Haller failed to succeed because of using the vegetative organ-systems for the same purpose. But to establish a "natural system" of plant-geographical types, as Humboldt intended to do, only the analogue characters of the vegetative organ-systems are important, because particularly by the adaptable faculties and functions of the organs for nutrition, sensation and motion the organisms are realizing their relations with the environment, which, particularly for the Plant Kingdom, are mostly identical with the corresponding geographical climatical zones and plant formations. So the "general types" in which Humboldt is interested in his Plant Geography, are the analogue-ones, which today we also call ecological types. Therefore, Plant Geography has the general task to investigate "*ob man unter den zahllosen Gewächsen der Erde gewisse Urformen entdecken oder ob man die spezifische Verschiedenheit als Wirkung der Ausartung und als Abweichung von einem Prototypus betrachten kann.*"¹⁴ This is very true typology of the analogous types, which corresponds logically and exactly with Cuvier's famous typology of the homologous types. The same way as Cuvier in his famous struggle with Geoffroy St. Hilaire, and in which Goethe, too, was deeply involved, was interested to find out if all types of animals could be derived typologically from one and the same fundamental type—from one "*Urtier*" as Geoffroy and Goethe postulated—or if we would have to consider different fundamental and autonomous types to cover the multiplicity of animal types, as Cuvier stated, that is also here the question for Humboldt's typology of analogous geographical or ecological plant formations. So Humboldt, too, here faces the problem: whether all these different plant geographical formations could be typologically derived from only one fundamental plant formation, or if it would be necessary to accept different independent ecological types. His answer is the same as Cuvier's, namely, in favor of a plurality of different fundamental ecological types. That we have to deal here with true typology and not with what we today call phylogeny, becomes perfectly clear if we consider only Humboldt's statement related to the corresponding animal geography: "*Die kleine und schlanke Form unserer Eidechse dehnt sich im Süden zu dem kolossalen, schwerfälligen, gepanzerten Körper furcht-*

¹³ "The basic force of organization holds all plant and animal forms in fixed unchanging and recurring types. This is true in spite of a certain degree of freedom for exceptional developments by the single part.

¹⁴ "... whether one can discover definite basic forms beneath the countless sorts of life growing from the earth, or whether one can observe the specific differentiations as a result of the breakdown and modification of *one* prototype only."

barer Krokodile aus. In den ungeheuern Katzen von Afrika und Amerika, im Tiger, im Loewen und im Jaguar ist die Gestalt eines unserer kleinsten Haustiere nach einem grösseren Masstabe wiederholt."¹⁵ This of course, is not historical phylogeny but only very true typology.

The analogous fundamental types of Humboldt's plant geography are called "*physiognomische Grundgestalten*"—physiognomic groundforms. Humboldt's main problem now is to make a typological "natural system" of them. But to do that we must first establish a fundamental law or general principle upon which may be based our physiognomic "natural system." In Humboldt's case this fundamental law, of course, has to be the basic principle of all Plant Geography. To have discovered it, remains for all times one of the best achievements of Humboldt in science. This basic principle of plant geography establishes an exact relation between the vegetation of a zone and its climate. So Humboldt's law states first that *equivalent medium temperatures create forms of vegetation, which are physiognomically analogous*, and second, that *with increasing altitude of the mountains or with increasing approach to the poles of the globe the tallness of the stem-organs decreases*. Based upon this principle Humboldt distinguished 17, later 19, physiognomic-typological groundforms of the vegetation. The most characteristic ones are the following: the bananatype, the palmtree, the treeferntype, the aloetype, the coniferoustype, the mimosatype, the lilytype, the cactustype, the grass- and reedtype, the mosstype, the lichentype, the mushroomtype, and others. Using these physiognomic types we are now able to compose them into perfect natural "genera" and "families" of vegetation-formations. Two of these Humboldt has studied especially during his Latin American voyage: the Llanos of Venezuela and the Hylaea Amazonica. The Llanos represents a holistic composition of grasses, mimosas and palms as its determining physiognomic groundtype, whereas the Hylaea, as a tropical rainforest, is defined physiognomically by trees belonging to the mimosa and laurel groups associated with palms, bamboos and heliconias.

Our considerations and remarks are surely sufficient to illustrate the perfect and almost exclusively typological character of Humboldt's Plant Geography in the sense of Goethe's general morphology. And Humboldt's Plant Geography, as his most important and significant scientific creation, is absolutely representative of all his scientific work. The physiognomic groundforms represent, in Humboldt's typological plant geography, the "general types" of Goethe's morphology, whereas the different "genera" and "families" of the vegetation formations—such as the Llanos or the Hylaea—are completely based upon the "compensation principle" of Goethe. If we compare, for example, (and like all typological morphology Humboldt's Plant Geography is a comparative science!) the amazonian Hylaea with an African tropical rainforest or the Venezuelan Llanos with an Asiatic tropical savanna, then in both cases we have the same "genus" or "family" of vegetation formation, but in each case composed of very

¹⁵ "The small, slim form of our lizard, in the South dilates into the huge, awkward, mailed body of the frightful crocodile. The form of one of our smallest house pets is repeated on a greater scale in the enormous cats of Africa and America, such as the tiger, the lion and the jaguar."

different plant and tree species, which this way have to *compensate* each other, but under the holistic rule of always retaining the same constant physiognomic vegetation formation. In a letter to his friend Berghaus Humboldt describes the compensation in different formations of plants in the following words: "*Die Einheit der Natur ist dergestalt, dass sich die Formen einander nach bestehenden unwandelbaren, noch nicht durch die menschliche Einsicht ergründeten Gesetzen ausgeschlossen haben. Kennt man auf irgend einem Punkte des Erdrundes die Zahl der Arten einer grossen Familie, z.B. der Glumaceen, Compositen oder hülsenartigen Gewächse, so kann man mit einiger Wahrscheinlichkeit sowohl die Totalmenge der phanerogamischen Gewächse, als auch eine Anzahl der Arten, woraus die anderen Pflanzengruppen bestehen, schätzen.*"¹⁶ The word "holistic" as used above means nothing philosophical but only scientific, namely that all our physiognomic groundtypes and formations represent true holisms but never mechanisms, as we have already explained these terms above. Humboldt, like Goethe, is using in this connection the platonic and aristotelic concept of *harmony*, which belongs always only to holisms but never to mechanisms, which are only able to produce physicochemical equilibria instead of living harmonies. These considerations guide us, then, to our last remarks, concerning Humboldt's physiological ideas and research.

IV

Humboldt's main work concerned geography in the broadest sense of this science. He was not only the creator of modern scientific geography, he also first founded Plant Geography as a morphological-typological science in the sense of his master, Goethe. All these morphological sciences deal with holisms, whereas modern causal physiology is always concerned with the research of mechanisms. Therefore if we now consider Humboldt's kind of physiology, we have to examine first its relation to his holistic natural philosophy.

Like Goethe, Humboldt too was always thinking physiologically. He surely was a typological morphologist, but he was always more interested in the functioning or in the *analogous* correlations of his types than in their pure typological constitution or homologies. When he was still a young student of only 19 years he wrote a letter to his brother Wilhelm, in which he speaks of the necessity to investigate more the physiological energies of the plants—its "*Kräfte*"—than its taxonomical constitution only. He says that such studies in the future will be of greatest practical importance for human nutrition. To study Botany this way would be for him essentially: "*weil ich an einem Werke über die gesammten Kräfte der Pflanzen ... sammle, ein Werk, das wegen des vielen Nachsuchens und der tiefen botanischen Kenntniss bei weitem meine Kräfte übersteigt, und zu dem ich mehrere Menschen mit mir zu vereinigen strebe.*"¹⁷

¹⁶ "The unity of nature is such that the forms are imposed one upon the other as enduring and unchanging, not yet derived from scientific laws imposed by man. If one knows exactly the cipher of the species of a large family—for example the Glumaceae, Compositae, or pod-forming plants—he could estimate with great probability the total quantity of phanerogamic plants which belong to the same group as the former."

¹⁷ "I collected into one work [everything] concerning all the forces of plants, a study which exceeded my own efforts because of the many inquiries and far reaching botanical knowledge, and a study in which I strove to unite more men with me in my effort."

So we see that his faculty to organize what we call teamwork today awoke early in Humboldt.

Thinking this way in typological holisms we cannot but expect that Humboldt in his general philosophy of nature has also been a holist and neither a mechanist nor a vitalist. To document that we have an early treatise which Humboldt wrote for Schiller's *"Die Horen"* where it appeared in 1795, in Humboldt's 25th year of life. He published this essay, titled *"Der Rhodische Genius"* later also in all editions of his most popular and beloved book, the *"Ansichten der Natur,"* wherefore we must take it for granted that his viewpoint about the philosophy of living nature published here was never abandoned by him. Here Humboldt describes what was then called *"Lebenskraft"* (vital force) with the following words: *"In der toten unorganischen Materie ist träge Ruhe, solange die Bande der Verwandtschaft nicht gelöst werden, solange ein dritter Stoff nicht eindringt, um sich den vorigen beizugesellen. Aber auch auf diese Störung folgt bald wieder unfruchtbare Ruhe."*¹⁸ In this way Humboldt describes what we defined above as physicochemical equilibria only. But then he continues: *"Anders ist die Mischung derselben Stoffe im Tier- und Pflanzenkörper. Hier tritt die Lebenskraft gebieterisch in ihre Rechte; sie kümmert sich nicht um die demokratische Freundschaft und Feindschaft der Atome; sie vereinigt Stoffe, die in der unbelebten Natur sich ewig fliehen, und trennt, was in dieser sich unaufhaltsam sucht."*¹⁹ That is the kind of activity the principle of living harmony realizes. Using the word *Lebenskraft* to describe it does not mean vitalism. In his principal physiological book, *"Versuche über die gereizte Muskel- und Nervenfasern nebst Vermutungen über den chemischen Prozess des Lebens in der Tier- und Pflanzenwelt,"* from the year 1797 Humboldt rejects his *Lebenskraft* as a vitalistic principle, not considering it necessary *"eine eigene Kraft zu nennen, was vielleicht bloss durch das Zusammenwirken der im einzelnen längst bekannten materiellen Kräfte bewirkt werde."*²⁰ But such an antivitalistic statement does not mean that Humboldt has been transformed now into a mechanist. Not at all! He continues to be a holist as in his *"Rhodischer Genius."* Even that *"Zusammenwirken der im einzelnen längst bekannten materiellen Kräfte"* inside any living substance is so characteristically of the organismic world that there exists nothing comparable to it in any physiochemical system. The relation between the purely physical and the truly living is here thought of just the way Schelling thought of it when he stated: *"Nicht, wo kein Mechanismus ist, ist Organismus, sondern umgekehrt, wo kein Organismus ist, ist Mechanismus."*²¹ That is true holism, even in the modern form of it. We are of the opinion that there is only one total

¹⁸ "Inert activity characterizes unorganized matter as long as the bonds of physical attraction remain in force, so long as no third element penetrates in order to associate itself with the other elements. However, unproductive inactivity again soon follows this disturbing penetration."

¹⁹ "The composition of the same substance is different in plants and animals. Here the life-force enters as dominant in its sphere; it does not concern itself with the Democritic attraction and repulsion of atoms; it unites matter which rushes eternally in inanimate nature, and it separates that which would seem to be inseparable."

²⁰ "... to term a special power that which perhaps would result merely from the combined efforts of material forces which are separately well known."

²¹ "It is not correct to say: Where no mechanism exists, there is organism. Rather, the reverse: Where no organism is to be found, there is mechanism."

reality, which comprehends likewise the physical and the organic world. But the physicochemical principles and laws are not sufficient to cover also the organismic reality in its totality. So we have to research the principles and laws of the organismic reality in the same autonomous way that we did also in physics. But having once discovered the biological laws, we are able to derive from them by logical simplification the corresponding physical laws. The inverse way, intended by the mechanistic philosophy of nature, is impossible, but also the vitalistic thesis of an essential dualism between the physical and the organismic. That is holism, which almost generally has been accepted by the great scientists of Goethe's time, not only by Goethe and Humboldt, but also by Carus, Joh. Mueller, K. E. von Baer and many other naturalists. And here, to say it again, is the historical meeting point where our own present science and natural philosophy encounters anew the Goethe-Humboldt-Time.

Now we are sufficiently prepared to determine exactly Humboldt's position in the history of modern physiology. Since its foundation during the Renaissance epoch we must distinguish two main trends in the historical development of modern physiological thinking and research. One of them is absolutely new and original, created by the same spirit of modern causal-mathematical science which also shaped the modern dynamics and physics of Galileo and Newton. This is the new Physiology of Harvey, based, of course, upon the equally new analytical and elementaristic Anatomy of Vesalius. The general characters of this absolutely new modern physiology are the same which are also borne by modern physics, namely dynamic, analytic, elementaristic and causal-mathematic. To-day we call this group of sciences the exact-mathematical-sciences. To all of them is Kant's statement valid, that any true science comprehends only so much real science as its mathematics content. The most evolved principle to create this kind of science is the "causal mechanism" mentioned above.

For Humboldt, as a holist, this kind of physiology was not, of course, convenient. Only one of its main features did also suit him—the mathematical treatment of its problems. Unlike Goethe, who disliked the newtonian way of science, that is, the most intimate correlation and collaboration between mathematics and science, Humboldt belonged to the most ardent adherents of this modern synthesis of mathematics with the study of nature. Particularly by overcoming its very deficiency, Humboldt made Goethe's science capable of meeting our actual scientific situation and helpful to us in establishing our own new ideals of knowledge. But first we must outline the second historical trend of modern physiology, with which Humboldt has also been closely associated.

This kind of physiology we have to characterize as *comparative typological physiology*. Its roots go down into aristotelian biology; therefore it is still outside the reach of causal thinking, and because of its comparative proceeding, also free of elementarism. Since the Renaissance this classical typological physiology acquired a new aspect, as often occurs with old manners when they come into contact and historical struggle with absolutely new ideologies. From the new mathematical physics our typological physiology took over the dynamic character and behavior. So the static platonic and strictly kinetic aristotelian typology has become, since the Renaissance, a steadily and permanently always more truly dynamic science like modern physics. This becoming dynamic of an orig-

inally static structure we can recognize historically in Linné's famous distinction between "good," i.e. static, and "bad," i.e. dynamic, species and also in Goethe's concept of his "*Urpflanze*" as a really existing individual. But in Goethe's "general type" he acquired definitely the dynamic type, which since then has been completely and perfectly conceived by Humboldt and Cuvier.

This dynamic and comparative typological physiology has now become the second fundamental tendency, developing throughout the history of modern biology side-by-side with the absolutely new causal-elementaristic physiology. Both trends meet each other not seldom in the same important physiologists as in Haller and Joh. Müller. But our typological physiology is realizing its own independent development; it begins its modern dynamic way in Albrecht von Haller. In his principal work, the "*Mémoires sur la nature sensible et irritable des parties du corps animal*" (4 Vols., Lausanne 1756-60), he states that sensibility and irritability represent specific and autonomous faculties of the living substance, and for which there do not exist any corresponding properties in non-living matter. By these carefully exercised comparative physiological experiments, Haller defeated definitely the first mechanistic theory about the living substance, namely the theory of Borelli, Baglivi, and others, which undertook to prove that all animal organs are nothing but simple mechanical instruments: heart: pump; lungs: bellows; glands: sieves; teeth: scissors, etc. Haller's theory belongs to the true typological statements because it is only describing physiological functions as they are, by no means explaining them the causal way as the mechanistic theory from Baglivi really did it. In the following time other important physiologists, following Haller's method and way of consideration, stated, from more and more animal organs, that they too are exercising specific organismic faculties which do not exist at all in non-living matter. The final conclusions of all this comparative dynamic-typological physiology made Joh. Müller express his well known theory of the "specific energy" of all living substance in all its different structures from the simplest cell to the most complicated organ. Based upon this absolutely typological and by no means causal theory, any living structure exercises its own "specific energy", wherefore in the pure physicochemical reality no comparable processes exist. The fundamental faculty of all living specific energies is the first discovered by Haller: *irritability*. One can illustrate its function by a known example of Joh. Müller's: Living skin reacts to burning differently than dead skin. If dead skin burns, it is transformed by pure chemical processes into other chemical substances; but if living skin is burned, it reacts in the specific living way by inflammation, which finally brings about additional new living skin. Only organismic "specific energies" act this way, if they are irritated physiologically. This kind of typological comparative physiology is still continuing in our own days, its last significant presentation being that of Jakob von Uexküll's so-called "*Umweltlehre*" (Doctrine of the environment of the organisms). This part of comparative physiology represents nothing but the consequent continuation of Haller and Joh. Müller from the field of organ-physiology into the modern ecological physiology.

Humboldt also has his very definite position inside this line of physiological development and research, in between Haller and Joh. Müller. He describes the general task of his above-quoted book on physiology with the following words:

"Ich habe gesucht, in der nachstehenden Abhandlung alles zusammenzudrängen, was ich bisher über Reiz und Reizempfänglichkeit der sensiblen und irritablen Fiber beobachtete," and in his physiological biograph Wilhelm Wundt (Bruhns 1872) refers to Humboldt's results with the following words: "Mit der Untersuchung der reizbaren Pflanzen beginnend, entwirft hier Humboldt ein für die damaligen Kenntnisse umfassendes Bild der Reizungscheinungen in der ganzen belebten Natur. Würmer, Mollusken, Insekten, Fische, zahlreiche Amphibien, Vögel und Säugetiere unterwirft er der Vivisektion, dem galvanischen und mechanischen Reizversuch. Ueberzeugt von der inneren Uebereinstimmung aller Organisation, vermutet er, dass die Reizbewegungen der Mimose und anderer Pflanzen, wenn gleich der galvanische Reiz bei ihnen unwirksam bleibt, auf den nämlichen Ursachen beruhen wie die Zusammenziehungen der tierischen Muskelfaser."²² All this is indeed very true comparative-typological physiology. The idea of the "innere Uebereinstimmung aller Organisation" is nothing but another word for Goethe's "general anatomical type." How near Humboldt in his final physiological conclusions comes to the general principle of the "specific energy" of Joh. Müller is cleared up in the following remarks: "Ich fange von der Erscheinung des Galvanismus an, weil ich durch die Art, wie ich die Versuche anstellte, unwidersprechlich erweisen zu können glaube, dass der Stimulus in diesem wunderbaren Phänomen grössenteils von den belebten Organen selbst ausgeht, und das diese sich dabei keineswegs bloss leidend, etwa als elektroskopische Substanzen, verhalten."²³ This remark about the own activity of the living organs when they meet with irritations from outside refers exactly to Joh. Müller's "specific energy" whose fundamental faculty is, of course, vital activity.

The typological comparative physiology in all its presentations from Haller until von Uexküll still lacks the application of mathematical thinking and therefore the characteristic modern synthesis of being a mathematicised science. To become a mathematicised science any doctrine has to fulfill two different but of course intimately correlated indispensable requisites. First of all it has to present its facts as exact measurements following the postulate of Galileo "to measure what is measurable, and to make measurable that which presently we are not yet able to measure." Second, we have to establish a causal principle which allows the application of mathematical calculi on measured facts to derive from them new, unknown statements. The modern causal-analytical physiology has taken precisely this way in its historical development. Harvey has been the first to

²² "I have tried to bring together in the following study all that which I have observed concerning the stimulation and the susceptibility to stimulation of sensitive fibers. . . . Beginning with research on sensitive plants, Humboldt here sketches what was for that time a comprehensive picture of all living things which were capable of receiving stimuli. He subjects worms, mollusks, insects, fish, numerous amphibians, birds and mammals to vivisection in order to test for galvanic and mechanical response. Convinced of the inner agreement of all organization, he conjectured that the response of the mimosa and other plants depends upon the same causes as the coordinate functioning of muscle fiber in animals, also if the galvanic stimulus remains ineffectual for the plants."

²³ "I begin with the appearance of galvanism because I believe that in this manner I could prove without contradiction that the stimulus in this wonderful phenomenon arises in great measure from the living organs, and that this is in no way merely *passive* like electroscopic substance."

make measurable the then most significant basic phenomenon of all physiology, the circular movement of the blood, and Descartes has delivered the most important philosophy for modern science since its beginnings, namely his mechanistic metaphysics, which postulates the identity of physical and organismic reality, thus making possible the scientific treatment of all biological phenomena as essentially being only physicochemical structures too. As we know, this *causal* principle of the "mechanisms" has had the biggest success any philosophical idea in science has ever acquired by shaping not only modern physics, chemistry and physiology, but also the whole corresponding technology upon which all our actual human civilization is based. But we know also that the profound crisis of our social, mental and political life has one of its reasons in the historical fact that this classical mechanistic philosophy is by no means any longer able to cover the most urgent and imperative spiritual necessities of mankind. We need a new philosophy, able to deliver a new ideal of knowledge and a corresponding principle of causality for all science to replace the theoretically exhausted causal principle of mechanism without, at the same time, abolishing its really great and stupendous acquisitions.

Humboldt, being a holist, could not of course follow modern physiology in its mechanistic way. But its first requisite, to establish all its facts in form of exact measurement, of the so-called "constants of nature," has been a way to exercise science which corresponds intimately with Humboldt's own ideal of knowledge and in which he surpassed also his friend Goethe, as we have noted at different times. And not only in his particular physiological research concerning irritability problems did he apply measuring methods, whenever possible, but also in his beloved plant geography he introduced statistical mathematics. Perhaps the most famous botanist of Humboldt's time was Alexander Brown, who first investigated the geographical distribution of plant species and families in a given formation of vegetation, counting the species and obtaining in this way an exact correlation between the natural system of plants and its given geographical distribution in the different floral provinces and formations. This method suited Humboldt best and, in elaborating the results of his botanical research in tropical America he applied it in the description of some plant formations visited by him and Bonpland. He wrote also a particular treatise about this kind of "botanical arithmetic" as he called it, which appeared in 1817 under the title: "*De distributione geographica plantarum, secundum coeli temperiem et altitudinem montium, Prolegomena.*" That time this statistical treatment of the problems of plant geography did not provide us with important results, but that is only due to the small number of taxonomic species known during that time. Probably in the future the same statistical processes in biogeography will bring us very important knowledge intimately related to the results of modern geobotany and geochemistry (Vernadsky, *et al.*). In any case Humboldt has proved that combining typological physiology and morphology with exact mathematical treatment by measuring and statistics is absolutely not only possible but an urgent necessity.

To transform that way the typological comparative physiology into a new causal-mathematical physiology we need also the second mentioned requisite of modern mathematicised science, namely the invention of a new ideal of knowl-

edge with its corresponding causal principle. If the holistic philosophy of nature and its corresponding "causal principle" (Smuts) of "holisms" are able to guide us in that direction, the next coming future has to prove it. Essentially hereby would be, if the holistic ideal of knowledge will have the faculty to produce new holistic mathematical calculi able to synthesize holisms into mathematical theories. The mechanistic idea doubtless has fulfilled such a great task, having synthesized the mechanisms of nature in its grandiose new mathematical calculi of the infinite-simalanalysis and analytic geometry, autonomously shaped by its own power of knowledge. But actually they are no more sufficient to cover also the requisites of future science.

Historians are not allowed to make prophecies. They normally are too much attached to distinct epochs and tendencies of the past to have still a free and unbiased look into the future. All they have to do, and only they can do it, is to provide the men who are actively shaping the future with a better understanding of the past; for it is the past which determines the future much more than the immediate present time. In such a sense I am of the opinion that Humboldt's science will have a certain importance to guide us in shaping our own future in science and knowledge.

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Molecular Configuration, Synthesis and Gene Action

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The thoughts and observations expressed here are a logical result of Professor Patterson's having urged me to study the natural nutrition of the fruit fly in 1940. I will not elaborate on the sequence of events that transformed a fruit fly ecologist into a macromolecule ecologist, but I do want to make clear my indebtedness to him. Once he got me started, he washed his hands, and only hoped that something good might come of it. Although he was not always certain as to what I was trying to do (in which feeling I frequently had to join with him), he never faltered with his support and encouragement.

The search for organismal units of structure and function in biology has led us to the recognition of organs, cells, chromosomes, mitochondria, plastids, macromolecules, etc. All of these have the quality either of being directly observable with eye or instrument, or at least of being made known to us as physical entities by physical or chemical techniques which are known to be reasonably dependable. On the other hand, we have also encountered some difficult problems in this quest for units. The foremost of these is the search for the hereditary units which segregate in definite ratios. In times past these have been called factors (Bateson, 1909), differentiating characters (Mendel, 1866), genes (Johannsen, 1911), cistrons or recons (Benzer, 1957), etc. But the same general difficulty has been evident from the very beginning of genetics, when we attempt to apply precise definitions to these entities or concepts. It is nearly always found impossible to define satisfactorily what segregates by what is observed. Several significant contributions in this direction have been made. First we may cite the formulation of genotype by Johannsen (1911) whereby he essentially separated what is transmitted through the gametes from the determined characters. These are necessary terms for development of causal genetics as well as formal genetics. The term, gene, has proved very valuable for several reasons. It served to replace the older term, factor, which was used ambiguously. Gradually gene came to mean determiner that is transmitted from generation to generation, as opposed to what is determined. The gene theory gave it a locus on the chromosome and defined its boundaries by crossingover. By implication it became a unit of function because when it mutated it caused a specific change in the phenotype inherited as a unit change. For many years it has served as a satisfactory genetic unit—a unit of physical structure on the chromosome and a unit of function and mutation.

Various geneticists, particularly Goldschmidt (1951), have attacked the gene concept, usually on the grounds that position effect, in particular the variegated type, as found in *Drosophila*, would indicate that an order of organization exists on the chromosomes that makes the gene unit an unnecessary assumption. More recently the concept of the gene has come under even stronger criticism because

of the recognition of the undoubted widespread distribution of pseudoalleles. The phenomena of *cis-trans* position effect (Lewis, 1955), complementation (Woodward, Partridge and Giles, 1958), etc. taken together with the fact that closely linked pseudoalleles have similar or related functions have necessitated a complete reexamination of what we mean by gene (Pontecorvo, 1958). In fact it is now quite evident that the gene as a unit as defined by previous criteria particularly as a crossover unit probably does not exist. It is necessary to redefine the term or abandon it entirely.

It is my purpose here to point out some facts and theories and to attempt to indicate a possible new approach to the problem. Instead of taking the usual approach of starting in the nucleus and proceeding outward I believe it might be more rewarding to start in the cytoplasm, and work back into the nucleus.

PROTEINS

The question we ask ourselves is this: Can we use our present theories and understanding of protein structure and biosynthesis to help us understand the structure of genetic material and the consequences of its functioning? Implicit in this approach is the important concept that these macromolecules constitute units of function in the cytoplasm and that their specificity is in some way directly or indirectly conferred upon them by the genetic material.

First, what are the relevant facts about proteins and their structure, function and biosynthesis? In summary this seems to be the picture. Proteins are macromolecules of molecular weights ranging from several thousand to about a million. They are polypeptide chains of amino acids with secondary foldings. They function as "work horses," as enzymes, transporting agents (for example, oxygen or electron carriers), regulatory agents, (for example, hormones) or antibodies. In these functions they show a remarkably high degree of specificity of function. It now appears probable that part or all of this specificity basically resides in the sequential order of the amino acid residues that make up the chain. These sequences in turn probably determine the types of folds held in place by disulfide and hydrogen bonds which give the protein molecule its active configuration. Hence, the different orders of the 20 odd amino acids known to be involved in proteins can theoretically account for the many different kinds of proteins which exist or have existed. One important detail in this connection is that those proteins from a single source, such as insulin, ribonuclease, etc., in which amino acid sequences have been determined exist as chemical entities of a single species represented by a single type of order, and not as populations of different but related species of molecules. This does not mean that mixtures of protein species may not exist, such as in heterozygotes, but simply that within each species homogeneity is practically absolute. Nor does it mean that families of related proteins do not exist. It is clear that they do. Insulin from cow, sheep, horse or pig all have slight differences in amino acid order, but yet they are all obviously related insofar as they all have the physiological effect of insulin and all have identical amino acid sequences in the rest of their chains (Harris, Sanger, and Naughton, 1956).

How is the function of a protein molecule related to its structure? This is a

problem still under extensive investigation. It is clear that many proteins, particularly those that act as enzymes or carriers, are associated with prosthetic groups or cofactors such as organic coenzymes, metal ions, or porphyrins such as heme. These non-protein groups are of extreme importance in determining the activity of the total protein-cofactor complex. But in addition to the cofactors, other aspects of the functional molecule complex must be important. For one thing, the same cofactor may be involved with a number of different proteins to produce as many different enzymes or carriers. The protein portion is basically an important part of the complex, therefore, since it endows the molecule with its specificity. It is generally assumed that this portion has a region or center called the "active center" which is most closely related to the relevant function of the molecule. This may be the site at which the coenzyme attaches, plus contiguous areas which dictate specificity of function. The active center may be a relatively restricted portion of the molecule surface or chain. It appears that in many cases parts of the chain of a biologically active protein are expendable when tested *in vitro*. For example, only one-third of the papain molecule is necessary for its activity. Up to 120 of its 180 residues may be removed without loss of activity (Hill and Smith, 1956). Also, as stated above, certain substitutions may be made in the insulin chain without altering the general function. The differences between hemoglobin A, S, and C discussed below are another example.

What is the function of the part of the protein molecule not included in the active center? It has been argued that the residual part of a protein is (1) a handle or holdfast which attaches a molecule in its proper position within the cell, (2) a necessary addendum as an energy transfer device, (3) a means to maintain stability and specificity of the active center (Steinberg and Mihalyi, 1957), or (4) a non-essential residuum formerly functional but now merely an appendix which may provide clues to the molecule's evolution but otherwise unimportant (Anfinsen and Redfield, 1956). There may be an element of truth in each of these, but I would add a more general statement already anticipated by others. *The residual part of the polypeptide chain acts as a buffer between the active center and the environment of the cell.* This includes the external and internal environment of the cell, and, within the cell, includes molecules ranging from inorganic ions to proteins and other macromolecular constituents. Merely having an active center is not enough. The active center must be protected from inhibitors which are otherwise natural and necessary constituents of the cell. The residual chain may do this by the way it is folded adjacent to the active center and the way in which it is folded would be directly related to the amino acid sequence in the chain. The substitution of a single amino acid in a chain of one hundred could conceivably alter the configuration of the entire molecule.

GENETIC IMPLICATIONS

Several conclusions may be drawn from the above observations relative to gene action and phenotypic expression provided one is willing to make a number of reasonable assumptions. If we assume that the total protein part of an enzyme or carrier is necessary under actual cellular conditions, and that a large part of it is really an environmentally selected-for buffer, we have at hand an explanation

for allelic gene differences, and the action of suppressors, enhancers, inhibitors, dilutors, and various unnamed modifiers in general. Present research strongly indicates that a gene mutation can modify the structure of an active protein by alteration of its amino acid sequence. This may involve only the substitution of a single amino acid. Such a change can conceivably occur within the active center, or in the residual part and may result in a protein which is worthless under any conditions. On the other hand, the new protein may be active, but only under certain conditions of the environment.

The hemoglobin studies of Ingram (1957) (Hunt and Ingram, 1958) indicate that the mutant hemoglobins C and S each differ from the normal A by the substitution of a single amino acid residue. In that position in the globin chain at which is found a glutamic acid residue in A there is substituted a lysine residue in C and a valine in S. Both C and S function poorly as oxygen carriers in the red blood cells, but for S, at least, the oxygen dissociation curve determined at 20° C and pH 7 is the same for the purified hemoglobins from both S and A individuals (Jeffries and Allen, 1951). This indicates that the active center has not been affected drastically, but that under certain environmental conditions, i.e., within the intact red corpuscles, the active center of hemoglobin S is not as efficient as the active center in A, but under other conditions it may be just as active. This may be due to a change either in the active center or in the residual part.

Turning to proteins with enzyme activities we find a number of examples of apparent enzyme alterations accompanying a single gene change. Yura (1959) reports that the enzyme, pyrroline-5-carboxylate reductase, (which catalyses the conversion of pyrroline carboxylic acid to proline), is present in a mutant of *Neurospora* blocked at this step in the biosynthesis of proline. The activity of this enzyme in the mutant, however, is about 30-fold less than the wild type. No significant difference was found between the enzyme from the mutant and the wild type with respect to relative affinity for the substrate, effective pH, or fractionation behavior, but they were strikingly different with respect to activation energy and thermostability. Mutant reductase has a temperature coefficient, Q_{10} , of 4.1, and a half-life at 48° C of 1.3 minutes. These values differ significantly from a Q_{10} of 1.6 and a half-life of 21 minutes found for wild type. The three enzymatic differences, low relative specific activity, high Q_{10} and low temperature stability, segregate as single gene differences with the phenotype prolineless.

Horowitz and Fling (1956) and Gest and Horowitz (1958) have demonstrated a difference between two tyrosinases in *Neurospora* apparently inherited through a pair of allelic genes T^s and T^L . T^s determines the presence of a stable tyrosinase, and T^L a thermolabile tyrosinase. In addition, the thermostable and thermolabile enzymes appear to be quantitatively different with respect to stabilization by Na^+ and K^+ . Both the thermostable and the thermolabile enzymes are formed in $T^L + T^s$ heterocaryons.

Maas and Davis (1952) and Fincham (1957) have found significant differences in enzymes formed by strains which reverted from the mutant to a strain similar but not identical to wild type. Their results cannot be as clearly interpreted as those given for the reductase and tyrosinase, but they, along with numerous observations such as those made by Stadler and Yanofsky (1959) on tryptophan revertants in *Escherichia coli*, make it evident that allelic genes prob-

ably produce qualitatively different enzymes or proteins in general. Whether these are active or not depends upon environmental factors. For example, a thermolabile enzyme would be ineffective at a high growth temperature but effective at a low temperature (Maas and Davis, 1952); an inhibited enzyme would be active in the absence or in the presence of reduced amounts of the inhibitor, etc. (Wagner and Haddox, 1951).

Some of the work on the tryptophanless mutants of *Neurospora* supports this latter conclusion directly. Tryptophan synthetase, the enzyme which forms tryptophan from indole and serine, is inactive or absent in those mutants of *Neurospora* which require tryptophan for growth, and cannot use indole + serine to replace the tryptophan requirement. However, in many of the allelic mutants at the *td* locus, which apparently control the production of this enzyme, a protein is present which has the same immunological specificity as tryptophan synthetase isolated from wild type but none of its enzymatic activity. This indicates strongly that the mutant alleles are forming altered proteins which are non-functional in the *Neurospora* cellular environment in which they initially occur. These immunologically active but enzymatically inactive proteins are called CRM.

Suskind and Kurek (1958, 1959) extracted and purified the CRM substance, or a protein which fractionated with it, from a strain (*td*₂₄) which required tryptophan when grown at 25° C. At this temperature there is no detectable tryptophan synthetase in the crude extracts. However, after fractionation, a highly active tryptophan synthetase is obtained. This enzyme from the mutant is inhibited by an inhibitor present in both the wild type and other *td* mutants. Significantly, the mutant enzyme is ten times more sensitive to the inhibitor than is the wild type. The inhibitory effect can be simulated by Zn^{++} .

It is clear from this example that mutations of a gene may result in a variety of different molecules, all functionally related—if they have a function at all—but able to act only under certain specific conditions of the environment.

Observations such as these give us a clearer understanding of the possible differences between wild type and what Muller (1932) called hypomorphic and amorphic alleles. Amorphs produce no enzyme, or one which is completely inactivated by an inhibitor; hypomorphs produce enzymes which are partially inhibited, and produce less than the wild type phenotype. The dominant wild type produces an enzyme which is in approximate harmony with its environment and its potential activity is probably in excess of available substrate.

Turning now to the problems of gene interaction we can see immediately that the modification of the cellular environment by the mutation of one gene may have a drastic effect on the action of the product of another. Suppressor genes may modify the environment in such a way as to relieve the inhibition of an enzyme as in the mutant tryptophan synthetase discussed above (Suskind and Kurek, 1959). (See also Wagner and Haddox, 1951.) Such an interpretation is in agreement with the studies on the suppressed *td* mutants of *Neurospora*. Active tryptophan synthetase has been extracted and purified for a number of the suppressed mutants of *Neurospora* and found to have the same dissociation constants as the tryptophan synthetase produced by wild type. This might be expected if

the mutant protein was altered in an area of the polypeptide chain outside of the active center in the residual part.

Furthermore this interpretation fits the seemingly paradoxical fact that suppressors can be both exceedingly specific and non-specific. A suppressor may suppress the mutant action of a single allele or a number of the alleles in the series, but not all (Yanofsky and Bonner, 1955). On the other hand it may be quite non-specific and suppress the mutant action of a number of non-allelic and seemingly functionally unrelated genes (E. B. Lewis, cited in Wagner and Mitchell, 1955). These phenomena can be expressed by assuming that genes, allelic or not, produce different kinds of protein subject to inhibition by the same or different inhibitors. Mutation of another gene controlling one of the inhibitors can therefore effect one or more of the proteins produced by allelic or non-allelic genes. Similar lines of reasoning can explain the action of inhibitor, enhancer, etc. genes. There remains to do the actual work to test this hypothesis rigorously, but Suskind and Kurek have made a good start.

What does all of this have to do with the problem of the gene as an hereditary unit? If we assume that the total polypeptide chain making up a functional unit as discussed above is necessary for that function, then, we must further assume that whatever template pattern the protein sequence is derived from contains the necessary information to organize the synthesis of a long chain of amino acids in a precise sequence. The primary pattern is now generally assumed to be DNA in those organisms with both DNA and RNA. According to Crick and co-workers (Crick, 1958; Crick, Griffin and Orgel, 1957) probably a minimum of three adjoining nucleotides is needed to determine one amino acid, and there are twenty arrangements of three out of sixty-four possible triplets from four possible nucleotides which do not give "nonsense" when arranged linearly side by side. (That the number 20 is also the number of amino acids which make up the bulk of protein may be no mere coincidence.) If a triplet is required to determine a single amino acid, then a protein containing 250 amino acid residues would require a nucleic acid template of 750 nucleotides per strand. Since it is possible that the cytoplasmic protein is formed from a RNA template obtained from the DNA pattern, it would be required that each protein have both DNA and RNA nucleotide chains with three times more nucleotides than the number of amino acids in the protein chain.

If we assume that all of the protein chain is important to the functioning of the protein *in vivo*, then all the polynucleotide determining it is necessary, and its limits are dictated by the limits of the functional molecule in the cytoplasm. I would call this length of polynucleotide chain the gene.

At the time of pairing of homologous elements, breaks and interchanges may occur within this gene as well as between it and adjoining genes, but interchanges within the gene will not be recognized, unless there are at least two different triplets involved in the two synapsing chains. Breaks may conceivably occur between the nucleotides of a triplet as well as between the triplets; however, if the DNA or RNA is bound to protein as nucleoprotein most of the time, permanent breaks should be rare. When not bound to protein the triplets might be stabilized by the amino acids attracted to them which would allow breaks and interchanges between unlike but adjacent synapsing triplets only during the rare intervals

that amino acids are not present, and homologous breaks or very close breaks occur simultaneously.

If instead of using the classical interpretation of the origin of interchanges by breaks and refusion of strands of synapsing chains, we use the "copy choice" explanation (Stent, 1958) as applied to virus recombinations, we may come to a similar but not identical conclusion.

Stent (1958) has proposed that the Watson and Crick (1953) explanation of DNA replication must be modified. He proposes in its stead the hypothesis supported by data from virus and bacteria that a DNA double helix acts as a template for the synthesis of a single RNA polynucleotide which then peels off and carries all the information present in the DNA chain. This chain may be stabilized by an adhering protein. Another DNA helix is formed from this RNA rather than directly from the mother DNA. First, however, Stent proposes that identical RNA from the same mother DNA, pair in what he calls identical duplexes, presumably for purposes of stability. Another DNA helix is formed from one of the RNA strands of the duplex as the two strands peel apart. If two homologous but somewhat similar RNA's pair (as from two allelic DNA's) the DNA formed on the duplex has the choice (copy choice) of copying part from one and part from the other. This could result in recombinations which would be recognized, if at least two differences were present in the two RNA's in the duplex. Thus interchanges occur not directly between paired DNA strands, according to Stent, but indirectly through RNA.

This hypothesis has the merit of fitting viral and bacterial data well, because complementary classes are not recovered from a single synaptic event. It does not, however, in its present form fit the data from higher organisms such as fungi, where complementary crossovers are regularly observed resulting from a single zygote nucleus.

Although this hypothesis may not explain interchanges in the basic genetic material in organisms above virus and bacteria as well as crossingover, it may be of some heuristic value to consider it as a possibility in the cytoplasmic synthesis of protein. Classically, recombinations have only been thought to occur in the genetic material, but why should they not also occur in the cytoplasm? The reason for advancing this hypothesis, which has already been anticipated by Woodward, Partridge and Giles (1958) in a somewhat different form, are several. There are a number of phenomena currently known, but not understood, occurring in heterozygotes or heterocaryons which may be explained on the assumption that recombinations occur within functional units in the cytoplasm at the time of replication.

If we extend Stent's hypothesis for DNA replication from a RNA duplex to protein replication from similar duplexes in the cytoplasm then we have the basis for recombinations occurring in protein. We may imagine the following sequence of events. In heterozygotes there must be two kinds of DNA representing a particular locus, each corresponding to a different allele. Therefore, there must be two kinds of RNA formed which get into the cytoplasm. Three kinds of duplexes may be formed from these; two different identical duplexes each representing one allele, and a duplex with non-identical but homologous RNA's. Protein polypeptide chains may be visualized as forming on this "hetero-

zygous" duplex following one strand of a duplex at a time, but at times switching from one to the other in copy choice fashion. If between the paired RNA strands of the duplex, there exist at least two differences, then there should be at least one protein type produced by copy choice interchange which is not formed by the identical duplexes. Hence, from certain heterozygotes we should expect more than the two expected types of a particular protein species. Indeed we do find such "hybrid substances" in dove hybrids (Miller, 1954), blood serum heterozygotes in man (Bearn and Franklin, 1958), and rabbit (Cohn, 1958). Hybrid substances produced in the cytoplasm by this means could explain the type of allelic gene interaction such as exhibited by antimorphs (Muller, 1932) and mixomorphs (Wright, 1941). Also the phenomenon of overdominance (Hull, 1946; Crow, 1948) may find a possible explanation here. It is only necessary to assume that in the case of antimorphic interaction in heterozygotes the hybrid substance or substances formed are recombination proteins incapable of being active agents in the cellular environment, and furthermore that they inhibit the activity of the active unrecombined proteins by combining irreversibly with the substrate. This would explain why two different alleles may be active when each is homozygous, but less active or inactive in heterozygotes. Alternatively, overdominance could be explained by assuming that the hybrid substance is superior in activity to either of the unrecombined proteins produced in the pure form in the homozygous strains. Furthermore, the phenomenon of complementation in which two mutant "allelic" genes produce a wild type effect in heterozygotes would fit the explanation for overdominance. This could apply to complementation in both heterozygotes of higher forms and heterocaryons of organisms such as *Neurospora*.

RECONS AND CISTRONS

The scheme presented in the preceding paragraphs may do little more than add confusion to a now already confused situation. Only the accumulation of more data can be expected to partly clarify the situation. Meanwhile, it may be helpful to consider certain other phenomena and confuse things even more.

Coiners of terms and phrases have been extremely active since genetics invaded the domain of virus and bacterium, and physicists and biochemists arrived on the scene to enlighten geneticists. Some of the new terms undoubtedly fill a definite need, and will become a permanent part of the biological vocabulary. They have been necessitated primarily by the fact that recombinations can occur within the limits of the genetic unit called the gene. Benzer (1957) proposed the term *recon* which he defined "as the element in the one dimensional array that is interchangeable (but not divisible) by genetic recombination." Demerec (1956) uses the term *site* with more or less the same meaning as recon. I would consider site and recon synonymous, and to fit them into the framework given above I would postulate either that a site is a single nucleotide (or pair) of a nucleic acid chain, or at most a triplet. With respect to protein a site determines an amino acid in the chain. A gene then is made up of the necessary series of sites to produce a functional protein. Alleles at a particular locus are the result of differences in sites. Hence there may be "identical" or "non-identical alleles" (Demerec, 1956). Non-identical alleles may be expected to complement one another

if a functional molecule can be derived by interchange as discussed above. Identical alleles obviously would be, under this hypothesis, unable to complement one another. The term *cistron* (also coined by Benzer, 1957) was introduced, again with very good reason, to describe those situations in which closely linked genes with apparently related functions show the cis-trans position effect. A cistron is a group of pseudoalleles—genes which give a mutant phenotype when two are present in the trans configuration and heterozygous with their wild type alleles, i.e. when each mutant allele is on a different homologue of the chromosome pair. When both mutant genes are on the same chromosome, their respective wild type alleles give a wild phenotype.

A variety of explanations can be given to explain this phenomenon (Lewis, 1954; Wagner and Mitchell, 1955) which all boil down to the assumption that the adjacent genes are related in a sequentially polarized function and in order to carry out this function the wild type or effective alleles must be together. The cistron then may be looked upon as a supragenetic unit. Conceptionally, no difficulties are encountered by adding this supra unit to the functionally defined gene unit described above. However we are then faced with the paradox that complementation may occur within a gene so defined but not between genes which are elements of the same cistron. Obviously more facts are needed here to clarify the relation between genes and cistrons. However, if we use the idea that interchanges may occur by copy choice in the cytoplasm to produce "recombined" proteins, we may find a way out. *These interchanges are possible only between homologous gene products but not ordinarily between non-allelic genes or their products.* Interchanges between genes will only be maintained in the "germ line." As such they will be recognized as crossovers, products of gene conversion, or just mutations.

CONCLUSIONS

The thesis presented in this paper, that we can only define the gene functionally by its product, makes no pretense at including the solution to all our problems. It is, I believe, one possible working hypothesis to be considered among others. It does have the merit of being testable. This is also bad for its longevity, for hypotheses which cannot be tested often last the longest and attract the most attention at symposia.

The ancillary thesis that recombinations may occur at the cytoplasmic level by copy choice at the time of formation of active proteins has the merit of providing for a more flexible system in the cytoplasm. A number of protein types are capable of being produced—more than the previously assumed maximum of two in heterozygotes. These may be selected for under changing conditions of environment. Indeed their occurrence may be a possible explanation for the apparent selective advantage held by heterozygotes in populations. (See Patterson and Stone, 1952).

In conclusion I must point out that Haldane (1958) raises an extremely interesting point related to the above discussion. He suggests first that "the word factor be used for the cause of an observable difference which shows Mendelian segregation." He then points out that though we must consider the gene a ma-

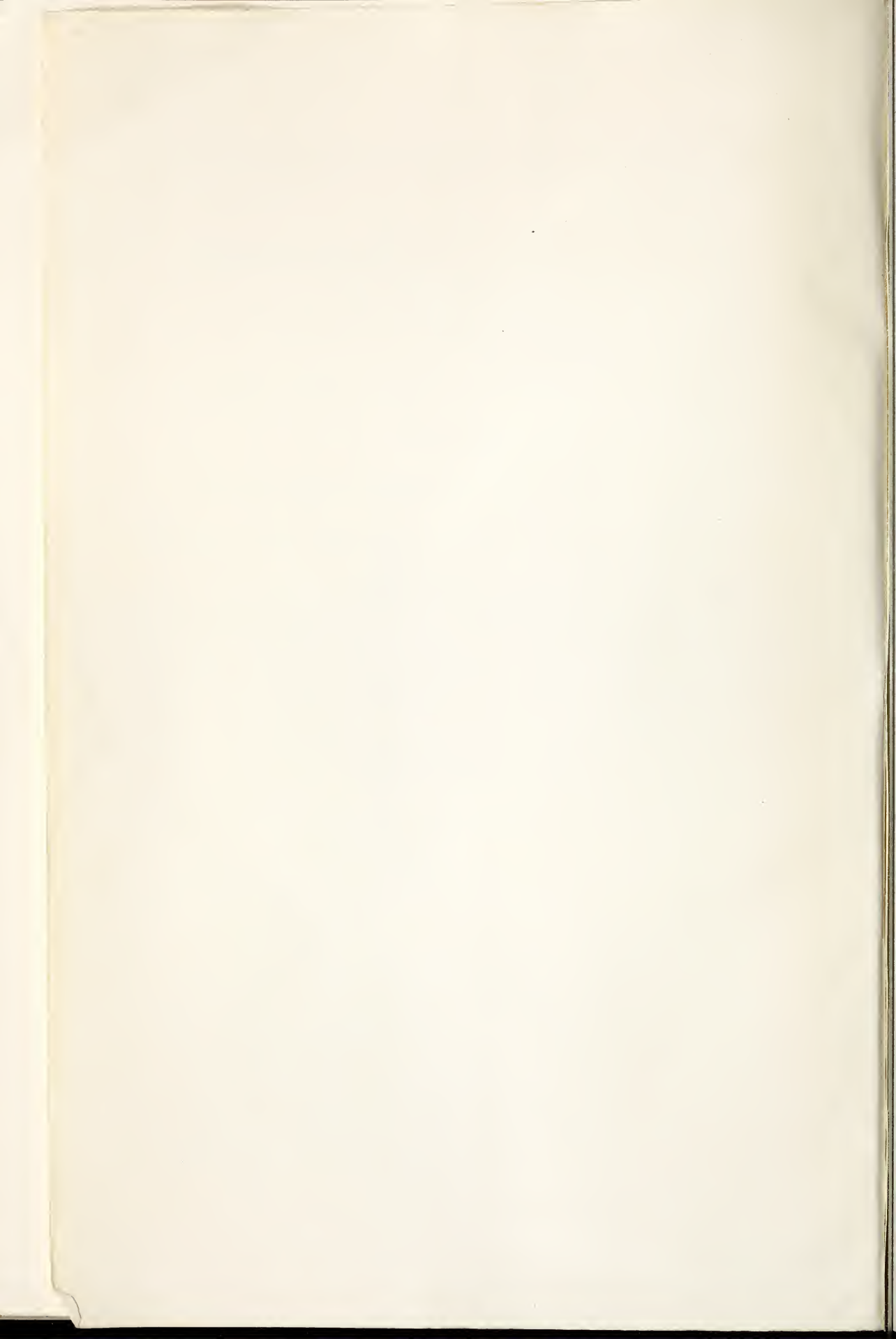
terial structure, we cannot localize it precisely. Its limits are certainly not exactly definable. A factor may be defined as a change in a few nucleotides by substitution or by loss or duplication of nucleotides. He then goes on to say "while then factors are units, but not necessarily or even usually real units, genes are not necessarily units. I do not go as far as Goldschmidt, and say that a gene can only be detected because it has mutated."

I do not agree with all that Haldane states here. We must focus our attention not on differences, which Haldane essentially identifies as factors, but on the real working units which give us the phenotype. If these are proteins, as discussed above, then their specificity must come from some source of information which we can define as that part of the genetic material which carries that sufficient information. This is the gene, and I think it is definable. Sturtevant's (1951) statement still has the ring of truth. "There is no escape from the conclusions that chromosomes are regionally differentiated, physiologically as well as visibly under the microscope; that particular and identifiable regions are necessary for particular reactions in the organism; and finally that these particular regions behave as units in heredity—specifically in crossingover." With the possible exception of the last phrase, "specifically in crossingover," it is my contention that this statement still stands as essentially true.

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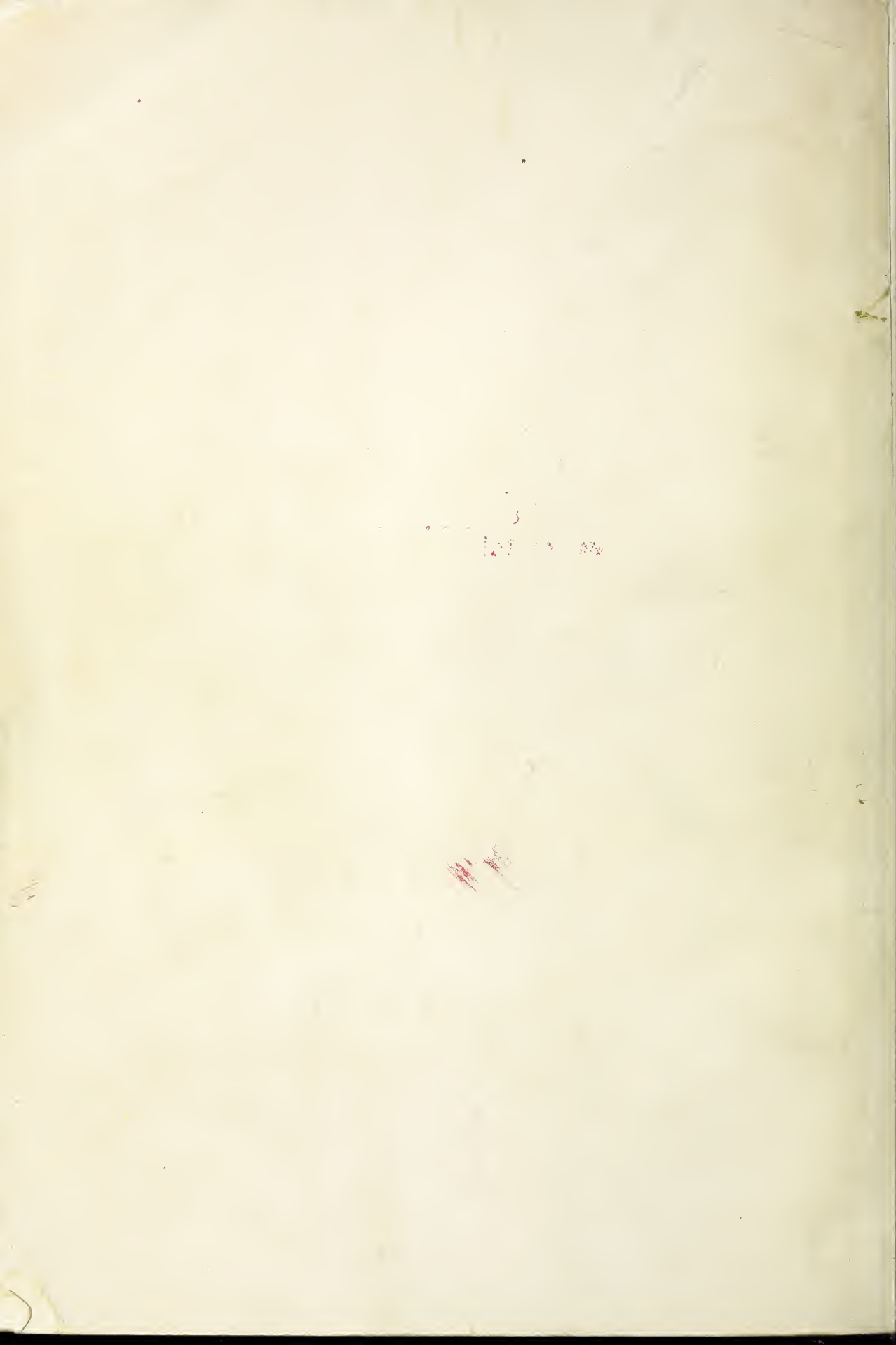
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